IN THE UNITED STATES DISTRICT COURT FOR THE
NORTHERN DISTRICT OF OKLAHOMA

W. A. DREW EDMONDSON, in his capacity as ATTORNEY GENERAL OF THE STATE OF OKLAHOMA and OKLAHOMA SECRETARY OF THE ENVIRONMENT C. MILES TOLBERT, in his capacity as the TRUSTEE FOR NATURAL RESOURCES FOR THE STATE OF OKLAHOMA,	)	
Plaintiff,	)	
vs.	)	4:05-CV-00329-TCK-SAJ
TYSON FOODS, INC., et al,	)	
Defendants.	)	

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THE VIDEOTAPED DEPOSITION OF VALERIE HARDWOOD, PhD, produced as a witness on behalf of the Defendants in the above styled and numbered cause, taken on the 18th day of July, 2008, in the City of Tulsa, County of Tulsa, State of Oklahoma, before me, Lisa A. Steinmeyer, a Certified Shorthand Reporter, duly certified under and by virtue of the laws of the State of Oklahoma.

Exhibit V

A P P E A R A N C E S

FOR THE PLAINTIFFS:	Mr. David Page Attorney at Law 502 West 6th Street Tulsa, OK 74119 -and- Mr. Louis Bullock Attorney at Law 110 West 7th Street Suite 770 Tulsa, OK 74119 -and- Ms. Liza Ward Attorney at Law P. O. Box 1792 Mt. Pleasant, SC 29465
FOR TYSON FOODS:	Mr. Gordon Todd Attorney at Law 1501 K Street, N.W. Washington, DC 20005
FOR CARGILL:	Ms. Leslie Southerland Attorney at Law 100 West 5th Street Suite 400 Tulsa, OK 74103
FOR SIMMONS FOODS:	Mr. John Elrod Ms. Vicki Bronson (via phone) Attorneys at Law 211 East Dickson Street Fayetteville, AR 72701
FOR PETERSON FARMS:	Ms. Nicole Longwell Attorney at Law 320 South Boston Suite 700 Tulsa, OK 74103

FOR GEORGE'S:	Mr. James Graves Attorney at Law 221 North College Fayetteville, AR 72701
FOR CAL-MAINE:	Mr. Robert Sanders Attorney at Law 2000 AmSouth Plaza P. O. Box 23059 Jackson, MS 39225 (Via phone)
FOR WILLOW BROOK:	Ms. Jennifer Griffin Attorney at Law 314 East High Street Jefferson City, MO 65109 (Via phone)

I N D E X

(Whereupon, the deposition began at 9:05 a.m.)

VIDEOGRAPHER: We are now on the Record for the deposition of Dr. Valerie Harwood. Today is July 18th, 2008. The time is 9:05 a.m. Would counsel please identify themselves for the Record? 09:05AM

MR. PAGE: David Page representing the State of Oklahoma.

MS. WARD: Liza Ward representing the State of Oklahoma. 09:06AM

MR. TODD: Gordon Todd representing Tyson Foods.

MR. GRAVES: James Graves representing George's.

MS. LONGWELL: Nicole Longwell representing Peterson Farms. 09:06AM

MS. SOUTHERLAND: Leslie Southerland for Cargill.

VIDEOGRAPHER: And on the phone?

MR. TODD: Folks on the phone want to identify themselves, please? 09:06AM

MS. GRIFFIN: Jennifer Griffin for Willow Brook Foods.

MR. SANDERS: Bob Sanders for Cal-Maine.

MS. BRONSON: Vicki Bronson for Simmons 09:06AM

Foods.

VIDEOGRAPHER: Thank you. The witness may be sworn in.

VALERIE HARWOOD

having first been duly sworn to testify the truth, the whole truth and nothing but the truth, testified as follows:

DIRECT EXAMINATION

BY MR. TODD:

Q Good morning, Professor Harwood. How are you? 09:06AM

A Fine, thanks.

Q Good. Now, this is the third time you've given testimony in this case. You've been deposed previously and you testified at the preliminary injunction hearing. That's right? 09:06AM

A That's correct.

Q Okay. Just quickly, the same ground rules as we used before. I will attempt to ask clear questions and if you don't understand my question, please let me know so that you're answering the 09:07AM question that I'm asking. Okay?

A Okay.

Q And remember to give verbal answers so they can be recorded. And I will attempt to use technical terms correctly, but you obviously are 09:07AM

much more familiar with them than I am. So if you think I'm misusing something or you know I'm misusing something, let me know so the Record is clear. Okay?

A Okay. 09:07AM

Q Great, and if you need a break at any point, just let me know and I'll try to get to a stopping point as quickly as possible. All right?

A Thanks, uh-huh.

Q Great. In front of you is a copy of the 09:07AM report you submitted and we've already gone and marked that as Exhibit 1. Do you want to take a quick look at that and make sure it's the report you submitted in this case?

A Yes, it is. 09:07AM

Q Great. Let's just put that aside and we'll get back to that later. Because you've been deposed before, I'm hoping that we can take care of a good number of subjects by just quickly updating what you've done since the last deposition. So let me 09:08AM just run through some of that stuff first. You testified previously that your opinions in this case regard microbial water quality and microbial source tracking. Is that still the case?

A That's correct. 09:08AM

Q Okay, and you testified previously that you are not providing expert geological, economic chemical signature, medical or hydrological testimony; is that correct?

A That's correct. 09:08AM

Q And you were retained as a consultant to the law firm of Motley Rice; is that right?

A That's correct.

Q Okay. Have you received any funding directly from the office of the Attorney General of Oklahoma? 09:08AM

A No, I have not.

Q Now, apart from your -- the prior deposition and -- well, apart from the hearing, have you spent any time in the Illinois River watershed since your last deposition? 09:08AM

A No, I have not.

Q In general terms, Professor, could you summarize the work you've done in this case since your last deposition?

A Yes. Since the last deposition we have -- 09:08AM Roger Olsen and the CDM team has collected some more water samples. The North Wind Laboratory has done some more analysis on water samples, and I think that's about all we've done.

Q Okay. 09:09AM

A Of course, I've done some additional data analysis for the report.
Q Right, and you submitted a report?
A Correct.
Q We talked at your last deposition -- you talked at your last deposition a bit about fate and transport, and let me just run through some characteristics here, and I hope we can take care of these pretty quickly. Since your prior deposition, have you conducted any study of the fate and transport characteristics of any bacterium in the Illinois River watershed? 09:09AM
A No, I have not.
Q So you have not studied how bacteria is affected by temperature? 09:09AM
A No.
Q Desiccation?
A No.
Q Predation?
A No. 09:09AM
Q Osmotic pressure?
A No.
Q UV exposure?
A No.
Q pH balance? 09:09AM

A No.
Q Nutrient availability?
A No.
Q Have you studied how the movement of any particular bacterium in the IRW is affected by its size? 09:09AM
A No, I have not.
Q Its shape?
A No.
Q It's surface charge? 09:10AM
A No.
Q Location in the water column?
A No.
Q Presence of vegetation?
A No. 09:10AM
Q The media it's moving through?
A No.
Q Have you cultured the Brevibacterium that you identified through your PCR process?
A No. 09:10AM
Q Why not?
A There has been no need to culture the Brevibacterium.
Q Have you identified it any more specifically than to say it's 98 percent consistent with 09:10AM

Brevibacteria avium?
A No.
Q And if you haven't cultured, I assume you also have not studied its fate and transport characteristics? 09:10AM
A That's correct.
Q Now, what you refer to as the marker, the biomarker in your term, what you're actually referring to is actually the DNA sequence that's contained by the Brevibacterium; is that correct? 09:10AM
A That is correct. We're referring to the DNA sequence, yes.
Q Okay. For clarity, I'm going to attempt to be consistent referring to the Brevibacterium as the PCR Brevibacterium and the sequence as the PCR 09:10AM sequence. Will those terms make sense to you? I just want to distinguish the two.
A Well, it's really a DNA sequence, so I guess --
Q We can call it the DNA sequence. 09:11AM
A DNA sequence.
Q If I refer to that, then we're talking about what you would refer to as the biomarker?
A Yes.
Q Now, we previously discussed or at your last 09:11AM

deposition you discussed that when a bacteria dies, its DNA remains in the environment for some period of time after that. Do you recall that?

A Yes, it can remain for some period of time.

Q Do you know how long the DNA sequence at issue in this case can remain in nature apart from the Brevibacterium that carries it? 09:11AM

A Typically in nature, bacterial DNA is rapidly degraded within -- and it depends on the environment, but within a matter of hours to several days. 09:11AM

Q Okay. You said it depends on the environment.

A Correct.

Q What kind of characteristics affect how quickly the DNA degrades? 09:11AM

A Characteristics would include the amount of ultraviolet radiation. It would include the amount of pred -- or not predation but the amount of organisms that would consume that DNA because they'll use it as a food source. So it would depend on the trophic level. So in a more eutrophic nutrient dense environment, then that DNA would probably be consumed more quickly than in a more allegatory thick environment. 09:12AM

Q Can DNA move in the environment after the 09:12AM

bacteria that carried it had died, become inactive?
A DNA could be transported along with water, yes.
Q Could it move in any other way?
A It would not be able to be motile on its own. So it would have to be transported by the movement of water or some other matrix. 09:12AM
Q Okay. Let's talk briefly about sources of bacteria in the IRW. Since your last deposition, have you studied sources in the IRW, apart from poultry, of any -- of fecal indicator bacteria? 09:13AM
A I have not.
Q Okay. Has anyone associated with the State's case?
A Roger Olsen of CDM has done some work with bacteria in cow manure. 09:13AM
Q Okay. Are you familiar with the nature of his work?
A I have read his report, yes.
Q Have you studied any sources in the IRW, apart from poultry, of E. coli? 09:13AM
A No, I have not.
Q Okay. Of Enterococci?
A No, I have not.
Q Campylobacter? 09:13AM

A No.
Q Salmonella?
A No.
Q Any other bacteria?
A No. 09:13AM
Q Have you undertaken yourself to quantify fecal production levels by any animal in the IRW?
A No, I have not.
Q Have you undertaken quantification of bacteria loading from any particular source in the IRW? 09:13AM
A I have not.
Q Now, you submitted a journal article to the Journal of Applied and Environmental Microbiology; correct?
A That's correct. 09:14AM
Q And we were provided a copy of that a couple of days ago. You're on the editorial board of that journal?
A That's correct.
Q Okay. Have you discussed your article with 09:14AM any of your colleagues on that board?
A No, I have not. That wouldn't be -- you don't do that.
Q Okay. You submitted it on June 11, at least according to the cover E-mail; is that correct? 09:14AM

A Correct, uh-huh.
Q What is its status?
A It is pending -- it's in review, so that means that the folks who have received it to review, who are anonymous, are still reviewing it. 09:14AM
Q An article is reviewed before it's accepted?
A Correct, usually by two to three members of the editorial board and/or ad hoc reviewers who are not part of the editorial board.
Q Okay. Do you have any expectation as to when it might be accepted? 09:14AM
A Usually it's about two months, so I would think in August we will know something.
Q When you submitted the article, did you recommend peer reviewers? 09:15AM
A Yes. That's a common practice.
Q Who did you recommend?
A I don't remember. I'd have to look back.
Q Okay. Could you provide us with that information? 09:15AM
A Yes, I could, I think.
Q And you do not know who is reviewing your work; is that correct?
A No. It's anonymous.
MR. PAGE: Mr. Todd, I think it would be 09:15AM

helpful, because there's so much going on, if you could provide me at least an E-mail or something. I'm not asking for a formal discovery request, but if you could provide me with some written information about any documentation -- 09:15AM

MR. TODD: Absolutely. I intended to.

MR. PAGE: -- after the deposition, that would be helpful.

MR. TODD: Not a problem. We will.

MR. PAGE: Thank you. 09:15AM

MR. TODD: Sure.

Q I made a copy of a few pages from the draft article. In the interest of not burdening us with paper, I didn't copy the entire thing, and I just printed it out this morning, and I apologize for it 09:16AM not being stapled. Now, if you flip to Lines 251 through 254, which is on Page 12, you note at the bottom of this page, quote, correlation of the biomarker with E. coli and Enterococcus spp. provides a line of evidence of the human health risk 09:16AM associated with the runoff from poultry litter application to fields, although there is evidence that regrowth of these organisms is possible once they are introduced into the environment. Now, when you refer to regrowth evidence or evidence of 09:17AM

regrowth, what are you referring to?
A E. coli and Enterococci have the ability in some environments to persist for months, and there are some -- there is some evidence that they may actually multiply in some environments, especially in sediment, and the multiplication would be slow but it could have -- it could potentially occur. 09:17AM
Q Do you have any evidence that the Brevibacteria you identified through your PCR process might grow in the environment? 09:17AM
A No, I don't have any evidence of that.
Q Okay. If the Brevibacteria did grow in the environment, how would that impact its correlation with indicator bacteria?
A That's almost impossible to say because it would really depend on how the Brevibacteria responded to nutrients and environmental stresses. So I mean it could respond very differently than E. coli or Enterococcus. 09:17AM
Q If they responded differently to the same environment and they're in the same environment, how would that impact the correlation? 09:18AM
A Again, the factors are so complex that I'm having a hard time thinking about how they might respond, but certainly if one -- if one group was 09:18AM

growing under certain conditions and the other group was growing under other responses and those responses were or those conditions were occurring at different times, then you could get difference in growth patterns. 09:18AM

Q Okay.

A However, I do need to qualify that by saying that the evidence for Enterococcus and E. coli growth in the environment is for very slow growth, so we're not talking about increasing by orders of 09:19AM magnitude in the sediment.

Q Okay. Flip to I think it's the next page of your packet. It's Table 4 of your submitted report, and if you look in the second column, which is numbers of samples tested, you report in your 09:19AM article testing ten litter sample, ten soil samples, ten edge of field samples, ten river water samples and six groundwater samples?

A Correct.

Q Why did you limit the number of river water 09:19AM samples to ten instead of including all of the tests that the State has done?

A Well, keep in mind that this article was written I believe, and I'd have to refresh my memory, but I believe it was written about a year 09:19AM

ago, and so the strategy or the idea was that we used the samples that we had analyzed in the first round of PCR sampling because we had -- if you remember, we had several different groups of samples that were submitted for analysis, and so this was our first pass, and so we wrote the paper then based on this first pass of samples, and then are planning to do a follow-up later on with the remainder of the samples.

Q Okay. So when you say it was written a year ago, are you telling me that you were not editing until several months ago? 09:20AM

A Oh, yes, we were definitely editing it several months ago but, again, so when you start with a body of works -- this is a coherent body of work here. This is what you do in science. You have a coherent body of work. You publish that, and then you move on to the next stage. So the other samples were -- are conceptually for purpose of the publication in the next -- 09:20AM

MR. ELROD: John Elrod.

A -- in the next phase, which would be the next paper that we would we write.

Q Let me hand you No. 3. Professor, I've handed you what's been marked as Exhibit 3. Do you 09:21AM

recognize this document?
A I haven't seen or reviewed this document lately. It certainly looks like in the style of the -- as I said, I haven't seen this document or reviewed it lately, but I may have seen it in the past. I just can't state positively one way or the other. 09:21AM
Q Well, let me represent for purposes of the deposition that this was in your considered materials -- 09:22AM
A Okay.
Q -- that were produced. So this document -- if that's true, this was in your possession?
A Okay.
Q This document seems to list various tasks that are going to be performed by you or someone else associated with the State's case at some point. You don't have any idea who drafted this document? 09:22AM
A It certainly is in the style of the documents that would have come from CDM.
Q Okay. Do you have any idea when it would have been drafted? 09:22AM
A No, I don't. Continuation of Task 5.8 from the 2007 scope of work, so it must be post 2007, but really it's just not ringing a bell with me. I'm 09:22AM

sorry.
Q Okay. Well, let's walk through the various subtasks that are identified here because I suspect you are familiar with them. If you look at Subtask 1, it notes there that the State has collected or at least that 550 samples have been sent to North Wind laboratory; do you see that? 09:23AM
A Yes, I do.
Q And it notes approximately that 200 have been analyzed already. 09:23AM
A Okay.
Q Now, is that about the number of samples that were analyzed around the time of the preliminary injunction hearing?
A Yes, I believe so. 09:23AM
Q Okay. What criteria, if you know, what criteria were used in deciding which of the total set of samples to actually test?
A For the qPCR?
Q Right. This says qPCR of existing and new samples, so, yeah, we're talking about qPCR testing. 09:23AM
A Okay. So I'm really going to have to dredge my memory for this, but my recollection is that our first pass for analyzing samples was to start with some of the samples that were more proximal to the 09:23AM

poultry litter spreading, like the edge of field samples, and work our way outward in terms of less proximal from the poultry litter spreading for the surface water samples. We also wanted to have some variety of groundwater and surface water samples to test, and we also -- I believe that Roger Olsen made some sort -- some discrimination in some cases based on the principal component analysis scores of certain samples, and if I remember correctly, also we wanted to test some samples that were high in indicator bacteria concentrations and others that were low in indicator bacteria concentrations. So those are some of the criteria that we had for selecting certain samples. 09:24AM 09:24AM

Q Okay. Now, of the -- this document indicates -- in that paragraph under Subtask 1, it indicates that approximately 70 of the balance of the samples, the balance of the 550, will be analyzed by qPCR; do you see that? 09:24AM

A Yes, I do. 09:25AM

Q Why were all 550 not tested?

A It was based on the throughput of the laboratory, so simply how many samples could they do, and it was based on the -- really the question we were asking and were we satisfied whether it had 09:25AM

been answered or not.
Q Now, by throughput, you mean the speed with which they could do tests?
A Right, right, their ability to actually cover so many samples and then, again, by our knowledge of how well our questions had been answered by the distribution of the biomarker in the watershed. 09:25AM
Q And what was the question that you were trying to answer?
A The question was following the pathway of the contamination from the poultry litter to the fields, to the edge of field water samples, and then out into the watershed, and then the dispersion of the marker and its distribution throughout the watershed. 09:25AM 09:26AM
Q Okay, and so I take it then that based on the samples that were run and the results you got, you're confident that they demonstrate that the biomarker, the PCR -- sorry -- the DNA sequence is distributed throughout the entire watershed? 09:26AM
A Is distributed -- is distributed -- well distributed within the watershed and particularly around the areas of greatest poultry contamination or poultry production. Sorry.
Q Okay. Who made the decision then not to test 09:26AM

any more samples?
A Generally the decisions that we make on sample testing are done collaboratively. So I don't -- I wouldn't say that any one person made the decision not to test more. 09:27AM
Q Did you make a recommendation at some point as to whether more testing should or should not be done?
A On water samples?
Q Sure. 09:27AM
A I can't -- I can't recall. I know that I felt confident that we had done enough with this last round of testing.
Q One more question on this then. If we look at the list of additional samples that are planned to 09:27AM be tested on the bottom of Page 1 --
A Uh-huh.
Q -- of these, would you agree that only 30, the 10 existing water samples from recreational areas -- actually 34 I guess, 4 existing water samples from 09:27AM referenced streams and 20 existing water samples from streams or other existing water samples from streams, that those are the only environmental samples that are going to be run here?
A Oh, can you rephrase that? I kind of got 09:28AM

lost.
Q That was a complete mess of a question. It lists a number of samples here, and the first number of them from duck samples on down through WWTP which I take it stands for wastewater treatment plants, those are all fecal samples; correct?
A Correct, yes.
Q And so those would be tested for confirming the specificity of the assay; correct?
A Correct, yes, uh-huh. 09:28AM
Q And then at the bottom, there's a new cattle waste sample?
A Correct.
Q And that would be tested for the same purpose?
A Uh-huh. 09:28AM
Q And two above that -- well, one above that is bedding material?
A Right.
Q And why would you test bedding material?
A We were interested in ensuring that the poultry litter biomarker signal, the DNA sequence signal would not be found in uncontaminated -- fecally uncontaminated bedding material. 09:28AM
Q Okay, and then it says you would test five new litter samples? 09:29AM

A Yes.
Q And what would be the purpose of testing additional litter samples?
A That would be for the same -- oh, these would be contaminated litter samples. I'm sorry. I'm not sure if the new litter samples mean new contaminated litter samples or new uncontaminated litter samples. 09:29AM
Q Okay. If we assume it means new used litter samples, why would you test; why would you think there was a need to test five additional used litter samples? 09:29AM
A There was really a -- simply the ability or simply the confirmation of our previous results is what we were interested in obtaining.
Q Okay, and so the balance of the samples listed here, the water samples from referenced streams, recreational areas and other existing water areas from streams, those tests, those 34 samples would be to look for the DNA sequence in the watershed; is that correct? 09:29AM 09:30AM
A Correct, correct, and, of course, the referenced streams would be unimpacted or relatively unimpacted streams, yes.
Q Right, and those would be outside the watershed; is that right? 09:30AM

A And those would be outside the impacted areas, yes. So now I'm starting to remember this document now.

Q Okay. I've handed you, Professor Harwood, what has been listed as Exhibit 4, which is an E-mail. Let me just characterize it quickly. It's an E-mail chain between you and Dr. Olsen and Ronald French, and as you move down the chain, Jennifer Weidhaas and Tamzen Macbeth are also included. Did I pronounce Jennifer's name correctly; how is it pronounced? 09:30AM 09:31AM

A Weidhaas.

Q Weidhass?

A Uh-huh.

Q Weidhass. I was over Germanisizing it, and she and Miss Macbeth are at North Wind; correct? 09:31AM

A Correct.

Q Now, if you flip to the second page of this E-mail, the first complete E-mail there right after your contact information lists various number of samples, and as I read this, this shows the total number of samples that were sent to North Wind, samples from which DNA was extracted and samples that were analyzed. Am I reading it correctly? 09:31AM

A Do you mean this part here? 09:31AM

Q Yes, ma'am.
A Okay. So this is a number that they received. The extracted means the DNA has been extracted and prepared for PCR, and then the number of samples analyzed is the actual quantitative PCR, the samples to which quantitative PCR has been applied. 09:32AM
Q Okay. How was it determined which of the samples that North Wind was sent that they would actually extract DNA from and then analyze?
A Those were again -- Roger Olsen from CDM would communicate directly with North Wind about the samples that were going to be processed and to what extent they were to be processed and, again, it was based on our -- the coverage of the various sample types that we were interested in and their position throughout the watershed. 09:32AM
Q Would different criteria be used to determine whether a sample would have DNA extracted from it from whether -- that those samples would in turn be analyzed? 09:33AM
MR. PAGE: I'll object to the form.
Q I'll rephrase. Why would DNA be extracted from a sample but then that sample not analyzed?
A I don't recall that. I don't recall how we determined that. 09:33AM

Q To the extent that you are familiar with the number of samples that went to North Wind, does this look like an accurate accounting?
A Off the top of my head, it looks accurate.
Q You don't have any reason to dispute this? 09:33AM
A No.
Q Flip to the next page, if you would, Page 3 of this E-mail, and look at the very bottom chunk. This is an E-mail from Jennifer Weidhaas to you, copied to Dr. Olsen and to Tamzen Macbeth, and if 09:33AM you look at the third line down, I'm going to read a sentence into the Record. Just FYI, MAN-PC-7A did not amplify with qPCR. However, this one was not officially requested by CDM so we are not reporting it as such. What do you take that to mean? 09:34AM
A I'm not sure. I think MAN-BC-7A was the beef cow sample on which we had had a contamination event way back when, and that one was -- it was determined that it was contamination, so we had -- we got a spurious positive on that. I believe that's the 09:34AM MAN-BC-7A, and so she's telling me that they did it again by qPCR, that they tried to amplify it again by qPCR, and that it did not amplify as it should not have.
Q Now, would that result have been reported -- 09:34AM

would that have been included in your report?
A No, because the MAN-BC-7A, that was all reported back when the first analysis was done, and we talked about it in the hearing, that particular sampling. 09:35AM
Q Okay, but it sounds like here they've tested it again; right?
A Yeah. I think she ran it through again just to make sure we were getting no positives.
Q Okay, and this test would not have been included in the data that was reported to you officially? 09:35AM
A It doesn't sound like it, but I'd have to look and see if it was.
Q Are you aware of any other instances in which North Wind tested samples that weren't included in the official data reports? 09:35AM
A Not to the best of my recollection.
Q Let's move on to Subtask 2 back on I think it was -- 09:35AM
A Exhibit 3?
Q Yes. I should write the numbers down so I'll get them right. Subtask 2, which is on Page 2, refers to reference laboratory validation. Do you see that? 09:36AM

A Yes, uh-huh.
Q Now, what is the purpose of having another lab cross validate North Wind's work?
A The purpose of having another lab cross validate is to -- is to -- well, just that. In science -- in science cross validation by other groups -- independent validation of test results is a major -- is a way that we test the reliability of the assay. 09:36AM
Q Now, the E-mail we were just looking at refers to Mike Sadowsky? 09:36AM
A Uh-huh.
Q Is that who you retained to cross validate?
A Yes. Mike Sadowsky at University of Minnesota is working on this. 09:37AM
Q Okay. Who is Mike Sadowsky?
A Mike Sadowsky is a professor of microbiology at the University of Minnesota. He's one of the leading environmental microbiologists in the country. 09:37AM
Q When was he retained?
A I believe it was May 2008, May or June 2008.
Q Did you all work out your contracting issues?
A Yes.
Q Okay. Have you worked with him before? 09:37AM

A Yes, I have worked with Mike. I've worked with Mike mostly on -- I've not -- just to clarify, I haven't co-authored anything with him, but I have worked with him on a book and worked with him on various microbial search tracking and environmental microbiology panels, expert workshop panels and things like that. 09:37AM

Q Now, what exactly was he retained to do?

A Mike's laboratory is going to utilize the qPCR assay and cross test some of the same samples that North Wind tested. 09:38AM

Q They're not going to recreate the entire North Wind process?

A That's correct.

Q Now, did you -- I take it you spoke with him in person about this? 09:38AM

A That's correct.

Q And you explained your procedure to him?

A Actually -- well, I very briefly explained the procedure to him, and then the details of the procedure were -- are in the -- are in the standard operating procedure of North Wind that was sent to him. 09:38AM

Q Okay. Did you explain your results to him?

A He knows about the -- he knows we're using the 09:38AM

poultry litter biomarker in the watershed, in the IRW watershed, and that we're using it as a tracer or a marker for poultry litter contamination. I didn't go into depth explaining what we found beyond the fact that the qPCR assay seems to work really well. 09:39AM

Q And is he familiar with the context of this lawsuit?

A I wouldn't say he's familiar with it. I'd say he's heard about -- he's heard very briefly about the lawsuit but certainly not any of the details. 09:39AM

Q But he knows he's been retained to validate something that's being used in a lawsuit?

A Correct.

Q What materials was he given? 09:39AM

A Wow. The standard operating procedure of North Wind for the qPCR, the -- a set of samples that are coded that have no reference to source, and a plasmin, so a piece of DNA that has the biomarker sequence cloned into it so he can use that for a positive control. 09:40AM

Q How many samples was he given?

A Somewhere around 30 I believe.

Q Do you know which samples he was given?

A I can't tell you off the top of my head. I 09:40AM

know there was some fecal samples of -- from sewage treatment plants, some -- or DNA extracts from human sources, cattle sources, goose and duck sources, and then some environmental -- extracts from environmental samples, like edge of field samples, 09:41AM
water samples and soil samples, and then poultry litter samples as well, DNA extract from poultry litter samples. So just to clarify, he doesn't have any of the raw samples. He has DNA extracts from these samples that were extracted by North Wind's 09:41AM
lab.
Q Exhibit 5, as I read it, lists the samples that were going to be provided to Mr. Sadowsky, Professor Sadowsky I should say. Does this look generally correct to you? 09:41AM
A Yes.
Q What were the criteria that were applied to select which samples would be given to him?
A Well, we definitely wanted him to have some positive samples where we would expect -- where we 09:42AM
knew that we had quantified the biomarker, and we wanted to give him some -- and we definitely wanted to give him the non-target samples so that he could verify the specificity of the analysis, and then we
wanted to give him some water samples that had high 09:42AM

concentrations of the biomarkers, some that had low but detectable concentrations, and then some in which we had not detected.

MR. TODD: Could whoever just joined us identify themself? 09:42AM

MS. GRIFFIN: It was Jennifer. My phone dropped off.

MR. TODD: Okay.

Q You mentioned the plasmin that they used, which I think is the DNA extracted from the 09:42AM Brevibacterium?

A Correct. The plasmin contains the DNA that's amplified from the Brevibacterium, uh-huh.

Q Do you know whether a sample of that has been provided to the defendants? 09:43AM

A I do not know.

MR. TODD: I'll submit you something in writing to request that as well.

MR. PAGE: Well, I don't think she still has it, but I think there were a lot of samples 09:43AM provided.

MR. TODD: I'll check to see whether we have it and if not, I'll submit you something in writing.

MR. PAGE: Okay, because I think there was 09:43AM

quite a bit of North Wind samples that were already collected I think by your experts.

MR. TODD: Right. I know a bunch of samples were shipped to Dr. Myoda's outfit. I'm just not sure that they were given the extract of the DNA from the Brevibacterium. So we'll circle back on that. 09:43AM

Q What is the status of Professor Sadowsky's work?

A He's -- we have received some communication from him that the assay is running in his lab, and he's tested some of the non-target samples, the samples from other species, and found those to be negative. He's sampled all of the -- or he's tested all of the litter extracts and found them to be positive, and he's actually in the process of asking North Wind for some more DNA extract, so they need to send them more samples. 09:43AM 09:44AM

Q Now, when he's done with all of his work, is he supposed to submit a written report to you of some sort? 09:44AM

A I believe so.

Q Okay. Do you have any idea when you should expect that?

A I'm thinking -- well, he's off to Thailand 09:44AM

next week actually, but I'm thinking that we would have results at least sometime in August.

Q Let's look to Exhibit 3, Subtask 3, which, as I understand it, appears to be testing for Salmonella and Campylobacter in the IRW using a PCR assay. 09:45AM

A Uh-huh.

Q Has that been done yet?

A No, and we actually decided not to do that.

Q Why not? 09:45AM

A Basically expense and then we felt like we established the connection with the indicator bacteria.

Q Okay, and Subtask 4 just refers to technical memoranda summarizing the results of Subtasks 1 through 3. Do you know if any of those have been prepared yet? 09:45AM

A Those would not have been prepared yet.

Q Let's go ahead and turn to your report now, which you have as Exhibit 1 right there, and we're going to march through this page by page and hopefully get us all out of here at a reasonable hour. Let me direct you first to Page 3. Section 2 of your report here that starts by discussing waterborne disease, and while your report seems to 09:45AM 09:46AM

focus principally on bacteria, you also mention risk from waterborne viruses and protozoa; right?

A Correct.

Q Did the State test for any specific viruses in poultry litter? 09:46AM

A No, they did not.

Q Okay. How about in the watershed more generally?

A No.

Q Do you intend to offer any testimony regarding specific viruses associated with poultry litter that cause a health risk in the IRW? 09:46AM

A No.

Q Same questions for protozoa. Did the State test for any particular protozoa in particular? 09:46AM

A No.

Q In the watershed?

A No.

Q Do you plan on testifying about any specific protozoa? 09:46AM

A No.

Q You characterize the waterborne route here in Paragraph 6 as being one of the, quote, most common routes of disease transmission.

A Correct. 09:46AM

Q What do you mean by common?
A Common meaning one of the ways that people most frequently get sick.
Q How -- put that in percentage term. What's common? 09:47AM
A I'm sorry, I don't have a percentage off the top of my head.
Q What other routes would you say are common?
A Can you clarify the question? So what other routes are common for -- 09:47AM
Q Disease transmission.
A For disease transmission, sexually transmitted, airborne routes of transmission, foodborne routes of transmission would be among the most common, zoonoses from animals. Those are among 09:47AM the most common.
Q Okay. If you wanted to go find out how common one route of transmission is versus another for a particular bacteria or for a particular pathogen rather, is there a particular source you go to look 09:47AM at?
A That's fairly difficult. It depends on whether you are asking a question across the world or within the United States.
Q Let's say within the U.S. 09:48AM

A Within the U.S. generally I would go to the literature and see what I could find in there, and typically I would also go to the CDC, Centers For Disease Control.

Q Okay. I take it that the frequency of water-based transmission varies by pathogen? 09:48AM

A That's correct.

Q What diseases are more frequently or most frequently water transmitted?

A Do you mean in the United States -- 09:48AM

Q Sure.

A -- or do you mean in the world? In the United States our most frequent transmission would be -- Campylobacter is one of the very most frequent. Salmonella is frequent. We have the protozoa, 09:48AM Cryptosporidium in particular. The enteropathogenic E. coli are among the more common. Shigella is relatively common, and then there are a lot of viral pathogens as well.

Q Okay. Is -- say out of a hundred cases of Campylobacteriosis -- I'm going to slaughter that pronunciation at various times. Out of 100 cases, how many would you say are water transmitted? 09:49AM

A That figure I don't have off the top of my head. 09:49AM

Q Do you have a figure for Salmonellosis?
A Salmonellosis, no, I don't. Sorry.
Q Thanks. Bear with my awful pronunciations. You mentioned a few other diseases or pathogens. Cryptosporidium -- is Campylobacter more often or less than water transmitted than Cryptosporidium? 09:49AM
A Again, I'm sorry, I just don't have those percentages off the top of my head.
Q Okay. Let's move on. I've handed you what's been marked as Exhibit 6. Are you familiar with this article? 09:50AM
A I'm not recently familiar with it. I may have seen it in the past. It's old, 1999.
Q Okay. This is Paul Mead, et al, Food-Related Illnesses and Death in the United States on behalf of the Centers For Disease Control and Prevention, and if you look at page -- the fourth page of the article, which is Page 610, you'll see a chart which gives rates of foodborne transmission for various agents, and the fourth one down there is Campylobacter, and if you scroll across, you'll see foodborne transmission percent is 80 percent. Do you have any reason to disagree with that? 09:50AM 09:51AM
MR. PAGE: Object to the form, lack of foundation. 09:51AM

A Well, again, my hesitation lies in the age of this article. We know that Campylobacteriosis has more recently become a reportable disease and has more recently become something that physicians might seek to diagnose, so -- and so I'm not sure how relevant these figures are to the state of the science in 2008. Things change really quickly in microbiology and epidemiology. 09:51AM

Q Okay. So articles that are ten years old are not particularly relevant? 09:52AM

A It would depend on the context. Again, I'm just -- it's of concern because Campylobacter, again, I believe it's only been known for about twenty years. So this particular article, again, it depends on the context, so here simply that it's reporting rates of illness. It's -- the other hesitation I'm having is that, you know, there's a huge problem with underreporting of waterborne disease. So I'm not sure that, especially back at this time, that Campylobacteriosis would have been reported at the level -- well, I know it wouldn't have been reported at the level that it occurs but I'm -- 09:52AM

Q But you can't cite me a more recent article what you'd consider a more accurate, present-day 09:52AM

accurate rate?
A No, I can't off the top of my head. Sorry.
Q Do you think you likely looked at such an article in preparing your report?
A One that showed the relative rates of -- could you clarify that? Then what sort of an article? 09:53AM
Q Well, you testified that waterborne transmission is common and you told us here that Campylobacter and Salmonella are among the most common diseases for waterborne transmission. 09:53AM
A Correct.
Q So would you have looked at an article to substantiate your opinion that --
A That they were common, yes.
Q That they're common? 09:53AM
A Yes, yes.
Q And would we find such an article in your considered materials?
A Yes, but not necessarily that would relate foodborne versus waterborne rates. 09:53AM
Q Okay. Let's look on down this list and you see Salmonella, non-Typhoid Salmonella.
MR. PAGE: Mr. Todd, would you give me a continuing objection --
MR. TODD: Absolutely.

MR. PAGE: -- on this since you haven't established the foundation of this document with this witness?

MR. TODD: Sure. No problem.

MR. PAGE: Thank you. 09:53AM

Q Non-Typhoidal Salmonella, 95 percent?

A I'm sorry, where are we? Are we back on --

Q Yeah, the same list. 95 percent foodborne, do you see that?

A Yes. 09:54AM

Q Okay. What's your reaction to that number?

A Well, again, I would take it with a big grain of salt simply because with my knowledge of the vast underreporting of diseases of all these types.

Q Okay. Just taking waterborne transmission, in your opinion how many -- how often is a disease -- waterborne transmission, how often does it occur in, say, recreational water, such as a river, as opposed to something like a swimming pool or hot tub? 09:54AM

A Again, those percentages, I don't know. I haven't seen any percentages. 09:55AM

Q Can you ballpark it for me?

MR. PAGE: Object to the form.

A Not really. So I -- as you might be able to tell, I'm a little -- I'm always a little skeptical 09:55AM

of these percentages and assigning, attributing so much to one and so much to the other because I know how difficult it is to really do this epidemiology. So that's one reason I don't really keep those numbers in my head. 09:55AM

Q Okay. Do you think that it's -- that waterborne transmission of a disease is more likely or less likely -- is more likely to happen in a swimming pool or in a river?

A Well, that would depend on what type of disease. 09:55AM

Q Okay. Let's take Campylobacter. Is Campylobacter more likely to be transmitted from one person to another in a swimming pool or river?

A I would say in a river because you don't have the chlorine factor. 09:55AM

Q Is that the only factor that would affect that analysis?

A No, not the only factor, but it's the dominant one in my mind. In swimming pools you have chlorination, and so there -- to the best of my knowledge the main pathogen that one worries about in swimming pools is Cryptosporidium because of its resistant to chlorine, whereas with Campylobacter you don't have that resistance. 09:56AM 09:56AM

Q Okay, and would the same answer hold for Salmonella?
A That would be -- the line of reasoning would hold similar.
Q Okay. Are there any other factors -- you said that chlorine would be the dominant one. What other factors would you consider? 09:56AM
A Can you clarify as to what other factors I would consider in --
Q In determining whether it's more likely -- well, let me give you some constants. Let's say the same person who has Campylobacteriosis gets into either a swimming pool or a river and the same other person gets into the same swimming pool or the river. Is it more likely that Person A will give Person B the disease in a swimming pool or the river? That's the question I'm asking. What we're now up to is what other factors would you consider in telling me which one you think is more likely? 09:56AM 09:57AM
A Okay. So let me make sure I understand. You're talking about person-to-person transmission, so from one person to another of Campylobacter or Salmonella? 09:57AM
Q Sure.
A And saying would that be more likely to happen 09:57AM

in a pool or in a river?
Q I guess it doesn't necessarily matter that it's coming from a person, but take the same starting volume, same starting number of Campylobacters dropped into a swimming pool or river. 09:57AM
A Then to the best of my knowledge, it would be more likely to occur in a river, yes.
Q Okay, and what factors are you considering in reaching that determination? 09:57AM
A Again, the chlorination would be the main -- lack of chlorine in the river would be the main factor.
Q What other factors, even the ones you are discounting? 09:58AM
A I mean that's really the dominant one. I can't -- you know, the other factors that I'm thinking of that would dominate would be, you know, the microbial load and how much water the water the person is ingesting, that sort of thing. 09:58AM
Q Does the size of the body of water matter?
A Not if we're talking about the same concentration of bacteria in each case, the same amount per unit volume.
Q Okay. Now, I said the same amount. The 09:58AM

absolute volume that's put into the body of water?

MR. PAGE: Object to the form.

A I guess I'm having a little trouble following the hypothetical scenarios just because I'm thinking, well, I mean how -- it would depend on how far the people were from the source, for example. 09:58AM

Q Okay. Anything else that occurs to you that would be relevant to this --

A How much of the bacteria were protected by sediments, turbidity in the water. So there in the river they might be more protected by organic matter, sediments, et cetera, than if they're in the swimming pool. 09:59AM

Q Let me change locations on you. How frequently -- if you know, how frequently is Salmonella transmitted person to person just directly? 09:59AM

A How frequently? I don't know the answer to that. I know that it can be.

Q Okay, but do you think it's a regular occurrence or a rare occurrence? 09:59AM

A That would depend on the person's access to hygiene, for example, and their practicing of hygiene. Whenever there's a Salmonella outbreak, there's usually at least some cases of 10:00AM

person-to-person transmission, but there are usually less person to person than there is from the waterborne or foodborne, so I would say proportionally less but I can't give you a percentage. 10:00AM

Q Okay. Would the same hold for Campylobacter?

A To the best of my knowledge, yes.

Q Now, going back to your report, on Page 3 you refer to full body contact. What do you mean by full body contact? 10:00AM

A Full body contact would be when the person has their full body in the water and --

Q Including their head?

A Including their head, yes.

Q Okay. So head under water. You note the hundred thousand people using the IRW for recreation that Dr. Caneday calculated. 10:00AM

A Yes.

Q Do you have any idea how frequently full body contact occurs within those hundred thousand? 10:01AM

A No, I don't.

Q You also note in Paragraph 7 that the most frequent result of exposure is intestinal, such as enteric disease or gastroenteritis; do you see that?

A Is that on --

Q It's the first sentence of Paragraph 7.
A Yes.
Q What are you considering as exposure in that sentence?
A Exposure has a pretty wide range. It can range from ingesting the water by swallowing the water or by drinking it on purpose. It could be accidental ingestion by when you are playing in the water or get submerged suddenly, but exposure could also be aerosolization as if you are in a canoe and slapping water or playing, even play fighting in a canoe, something like that. So exposure has a pretty broad range. 10:01AM 10:01AM
Q So exposure really means any exposure?
A Yes. 10:02AM
Q Okay. Do most exposures result in illness?
A I would say no.
Q Okay. So when you say the most frequent result of exposure to waterborne pathogens is intestinal illness, is what you really mean the most frequent result of infection or ingestion of waterborne pathogens, not actually just exposure? 10:02AM
A Well, if there's an adverse -- what that means is if there's an adverse outcome, if there is an illness, it would be an intestinal illness. 10:02AM

Q Okay. I gotcha, I gotcha. The bottom of Page 3 there, I guess, four sentences up from the bottom, you refer to acute febrile respiratory illness.
A Correct.
Q And this is something you hadn't mentioned previously. Can I just call it AFRI for short? 10:02AM
A (Witness nods head up and down).
Q What is AFRI?
A I guess it's a bit like SARS, Sudden Acute Respiratory Syndrome, where -- and maybe I said that wrong. Anyway, I'm not saying it's SARS at all, but that you basically have upper respiratory symptoms; you have fever; you may have pneumonia or pneumonia-like symptoms. 10:03AM
Q Does it generally require hospitalization? 10:03AM
A I do not know that, the answer to that question.
Q Does it usually require medical attention of some sort?
A I don't know. 10:03AM
Q What microbes has it been linked to?
MR. PAGE: I'm sorry, I couldn't understand you, Mr. Todd.
Q What microbes has it been linked to? The testimony says that it has been linked in 10:03AM

epidemiological studies to elevated microbial pollution levels, and I'm just wondering which microbes.

A Well, so in this case what this statement was about was about the linkage between high indicator organism levels that indicate fecal pollution and their connection. So not linked to specific disease-causing organisms but to fecal pollution and their indicator, the Enterococci. 10:03AM

Q Okay. Have you studied any incidents of AFRI in the IRW? 10:04AM

A No.

Q Are you familiar with any incidents of it in the IRW?

A No. 10:04AM

Q Are you familiar with any incidents resulting from exposure to water in the IRW?

A No.

MR. TODD: We'll go ahead and stop and change the tape. 10:04AM

VIDEOGRAPHER: We're now off the Record. The time is 10:04 a.m.

(Following a short recess at 10:04 a.m., proceedings continued on the Record at 10:19 a.m.) 10:19AM

VIDEOGRAPHER: We are back on the Record. The time is 10:19 a.m.

Q Okay. Professor, you've mentioned a couple of times the underreporting of disease and you mentioned it in your report as well. Is it the disease itself that is underreported in that -- well, let me back up. With regard to this case, the diseases you will be discussing in this case, is it that the disease itself is underreported as in people or as in there is no public awareness that a certain person was sick, or is it that diseases are not specifically linked to water or perhaps both? 10:19AM

A Well, it's both in that frequently when people have gastroenteritis, they wait it out, they may miss a day or two or three of work and school, and they don't in their head specifically link it to this is some sort of a disease caused by a microorganism, and then even when people go to the doctor and even when the disease is diagnosed, it still doesn't end up being reported to the CDC in both cases. 10:20AM

Q Okay. Some of both. Have you ever yourself studied the underreporting of disease?

A No, I have not.

Q So you've never published anything on that? 10:20AM

A No.
Q On Page 4 of your report, you quote the World Health Organization, this little block quote here, and you quote, characterization of illnesses -- infections and illnesses due to recreational water contact as being generally mild; do you see that? 10:20AM
A Yes.
Q What do you take generally mild to mean?
A What I just described. So it's not mild to the person, but vomiting and diarrhea for two or three days, again, missing work and school, but then recovering on their own. 10:20AM
Q Okay, but seeking medical treatment or not seeking medical treatment?
A Frequently not seeking medical treatment. 10:21AM
Q Okay. You testified previously that plaintiffs have not undertaken any epidemiological study to quantify disease in the watershed. Is that still the case?
A Can you say that again? Sorry. 10:21AM
Q You testified I think at your last deposition that -- you were asked whether plaintiffs have taken any study to document levels of disease in the watershed.
A Correct. 10:21AM

Q And that still has not been done?
A Correct, it has not been done.
Q So the plaintiffs haven't conducted any epidemiological study to assess levels of Campylobacteriosis or Salmonellosis? 10:21AM
A Correct.
Q Okay. Have you yourself ever designed an epidemiological study?
A I have written a grant for an epidemiological study with the aid of epidemiologists, but myself am not an epidemiologist. So I'm familiar with the methods used, but I would seek help from an epidemiologist when design and study -- 10:21AM
Q You need to translate your field of jargon for me. You said you wrote a grant. Does that mean you got the grant and did it or proposed a project or -- 10:22AM
A This particular grant is a proposed project for an Environmental Protection Agency and the Florida Department of Environmental Protection, and the first phase of it is funded but the second epidemiology phase is not yet funded. 10:22AM
Q Okay. Now, you note -- this is in Paragraph 9 on Page 4 still -- that infants, children, pregnant women, elderly and the immunocompromised are more susceptible to waterborne infections. 10:22AM

A Correct.
Q Do you see that? Do you have any notion of the hundred thousand individuals who Dr. or Professor Caneday identified, any idea how many of them are infants? 10:22AM
A No.
Q Do you suspect there are many infants going for floats in the Illinois River watershed?
MR. PAGE: Object to the form.
A I really don't know. 10:23AM
Q Do you have any idea how many of the hundred thousand are children?
A No, I don't.
Q Pregnant women?
A No, I don't. 10:23AM
Q Elderly?
A No, I do not know.
Q Immunocompromised?
A No, I don't know.
Q Let's turn to the notion of bacteria that are in a viable but not culturable state, and this is something you discussed and testified about previously. Viable but not culturable does not mean undetectable; right? 10:23AM
A Viable but not culturable means undetectable 10:23AM

by conventional culture methods, but there are other methods that could potentially be adaptive for detecting them.

Q They could be detected, for instance, for DNA-based methods, such as PCR; is that correct? 10:23AM

A That's correct.

Q What are the -- what are the relative advantages of doing culturing instead of -- over PCR?

A The biggest advantage of -- well, I guess if you can clarify that a little bit, so you asked me what are the biggest advantages of doing culturing over PCR show. In what context are you referring to? 10:23AM

Q That's a good question. Which one is faster? 10:24AM

A PCR was faster.

Q Which one is cheaper?

A Oh, that depends on the method. So some kinds of culture method are cheap and some are not.

Q If the PCR assay is already developed, so science has been done and it's been verified and it's known to identify, say, Campylobacter, so that's all in the box and you pull it off the shelf and you are going to use it, is it cheaper to do that or culture? 10:24AM 10:24AM

A If there were out of the box methods for Campylobacter for PCR, it could potentially be cheaper, but I'm not aware of any.
Q You're not aware of any off-the-shelf Campylobacter PCR assays? 10:25AM
A Correct.
Q How about for Salmonella?
A Same thing. Again, I'm not aware of any -- and that would be for environmental samples. Obviously there are assays available for clinical 10:25AM diagnostics, but it's real different when you are working out of the environment.
Q Explain to me the difference between the two. My knowledge runs short.
A Clinical -- in clinical samples, the organisms 10:25AM tend to be at high concentrations because they're coming from feces, for example, or if you are really unlucky from blood, and in environmental samples, the targets tend to be more dilute and they also tend to be -- you tend to have problems with the PCR 10:25AM in terms of inhibition of PCR so you have to do a lot of sample cleanup.
Q But isn't the point of PCR that you can replicate -- you take a small starting quantity of DNA and replicate it to a point you can measure it 10:26AM

and identify it?
A That's correct.
Q So if you can control the inhibitions, the inhibiting factors, then you certainly could use a PCR assay on your environmental sample? 10:26AM
A In many cases you can.
Q When was the notion of the VBNC, when was -- for shorthand, when was this state identified in the scientific literature?
MR. PAGE: I'm sorry, I didn't understand the question. 10:26AM
Q Viable but not culturable, I was just going to refer to it as VBNC. Is there a shorthand for it?
A VBNC is a shorthand, yeah.
Q VBNC, is that -- 10:26AM
MR. PAGE: There was second part of your question I didn't follow. So do you mind? I apologize.
MR. TODD: No, no, not at all, and I'm happy to restate it. It was a mess. 10:26AM
Q When was the VBNC concept, this state, first identified in the scientific literature?
A I think it was around 1970 with Rita Caldwell's work.
Q Okay. So it's been around awhile. How long 10:27AM

have you been familiar with the concept?
A I've been familiar with the concept since graduate school, so 1990.
Q Have you ever yourself studied it?
A Yes, yeah. We're doing some work right now in my lab on viable but not culturable E. coli and Enterococci, for example. 10:27AM
Q What are you doing?
A We are assessing the extent to which the bacteria may persist in sediment samples in a viable but non-culturable state. 10:27AM
Q Are you doing that for this case?
A No.
Q Apart from the work you're doing in your lab right now, have you ever written about any bacteria's ability to enter that state? 10:27AM
A No.
Q When did you first consider the VBNC state in connection with this case?
A I would -- I would think it would be -- I would think it would be from when I started working on it, which I think was 2005. 10:28AM
Q Okay. Did you at any point suggest that in order to generate a more accurate count of pathogens in the IRW, it would be appropriate to use a test 10:28AM

other than just a culture-based test to identify it?
A We had some conversations about using PCR, and knowing the results that we were getting with the indicator bacteria and then moving toward the development of the biomarker, we just never went any further with the PCR tests. 10:29AM
Q Let's talk a little bit about Campylobacter. I take it, based on what you told me earlier, that the State hasn't done any additional testing for Campylobacter since your last deposition? 10:29AM
A Correct.
Q You note on Page 6 now of your report that Campylobacteriosis is usually limited to mild to severe gastroenteritis but that it can also result in Guillain-BarrT Syndrome and Reiter's -- is it Reiter's or Reider's? 10:29AM
A I think it's Reiter's.
Q Reiter's Syndrome. You say usually. Can you translate that into an incidence rate of one versus the other? 10:29AM
A I believe that Guillain-Barre Syndrome occurs in less than 5 percent of people that are diagnosed with Campylobacteriosis.
Q How about Reiter's Syndrome?
A Reiter's Syndrome, I'm not sure, but it's less 10:30AM

common that Guillain-Barre.

Q Since your last deposition has anyone associated with the State's case studied Guillain-Barre Syndrome in the IRW?

A Not to the best of my knowledge. 10:30AM

Q Are you familiar -- are you aware of any case of Guillain-Barre Syndrome in the IRW?

A No.

Q What is Reiter's Syndrome?

A It is -- you know, I can't say for sure. I'm sorry. 10:30AM

Q So you've never studied it?

A No.

Q Okay. Have you ever studied Guillain-Barre Syndrome? 10:30AM

A Not beyond reading articles, not specifically in my lab.

Q What you include in your report about the two syndromes, I take it, is just based on your literature review? 10:30AM

A Correct.

Q I take it -- are you aware of any case of Reiter's Syndrome in the IRW?

A No.

Q Are you aware of any case of Reiter's Syndrome 10:30AM

caused by exposure to bacteria derived from poultry litter?

A No.

Q Have you ever studied Campylobacteriosis itself as a disease? 10:31AM

A No.

Q Have you ever studied Campylobacter as an organism?

A No, not beyond literature review.

Q You mention, and this is Page 6, carryover to Page 7, you note antibiotic resistance in Campylobacter and Salmonella. Does antibiotic resistance vary geographically? 10:31AM

A That's such a broad question. I really would have a hard time answering it. Can you narrow the question down? 10:31AM

Q Sure. Would -- let's say that Campylobacter becomes 50 percent resistant to a certain antibiotic in a study in say, I don't know, Oklahoma. If I went and looked at Campylobacter in England, would I expect to find the -- could I expect to find the same resistance or could I draw no conclusion on the Oklahoma study as to what I would find in England? 10:31AM

A There are regional differences in antibiotic resistance patterns in both the pathogens and the 10:32AM

commensal bacteria than the relatively non-harmful bacteria. They're based in large part on the animal husbandry practices. So to the extent those practices vary regionally, then antibiotic resistance could vary regionally. 10:32AM

Q So it's possible there could be antibiotic resistance levels specific to the IRW based --given the poultry industry that's here?

MR. PAGE: Object to the form.

A I would doubt that there was antibiotic resistance specific to IRW simply from the knowledge that many of the poultry practices are carried through in large scale from the integrators, but there could certainly be regional differences in terms of if there is predominant animals in one region versus in another, then you might see differences in antibiotic resistance. 10:32AM 10:33AM

Q Has the State made any study of antibiotic resistance specifically in the IRW?

A No. 10:33AM

Q Has anyone associated with the State's case made any study of antibiotic resistance arising out of use of poultry litter in the IRW?

A No.

Q I'm going to shift to Salmonella. Have you 10:33AM

ever studied Salmonella as an organism?
A We have carried out studies in which Salmonella was one of our analytes that we detected, so, yes.
Q Apart from using Salmonella as an analyte -- let's back up. Do you mean by that you studied -- well, explain to me what you mean by that. 10:33AM
A We have tested water, water samples and sediment samples for the presence of Salmonella and confirmed their presence by PCR. 10:34AM
Q Apart from testing for presence-absence, have you ever studied Salmonella as an organism in terms of this is Salmonella, these are its characteristics, these are its qualities; have you ever conducted a test like that? 10:34AM
A No.
Q Have you ever studied Salmonellosis as a disease?
A No.
Q Okay. On Page 8 of your report you note that a transfer of Salmonella to poultry carcasses from intestines during slaughter. It's in Paragraph 17. Do you see the first sentence of Paragraph 17? 10:34AM
A Yes, I see that.
Q Okay. Now, that -- transferred to a carcass 10:34AM

during slaughter, that doesn't lead to waterborne transmissions, does it?

A No, it should not.

Q Okay. Now, you identify -- let's see where we are. In Paragraph 18 you note that Salmonella infections are frequently transmitted by the waterborne route, and you identify two studies in particular which regarded outbreaks. How -- when you say frequent, what do you mean by frequent; what is frequent in your mind? 10:35AM

A Frequent in my mind is when it's reported in the literature and in the CDC waterborne summaries as a major cause of waterborne disease.

Q What does major mean?

A Major would be one of the top five contributors to waterborne disease. 10:35AM

Q Okay. Would that hold -- you identify two what you characterize as Salmonella outbreaks here. Can you tell me the first of these, the Angulo study from 1997, can you tell me what happened in that incident? 10:36AM

A Oh, I'd have to look back at the papers. I don't have them at the tip of my fingers, brain.

Q How many Salmonella outbreaks have there been, say, over the last 20 years? 10:36AM

A I can't give you a number.
Q Okay. Let me give you the aforementioned study. Professor, I've handed you Exhibit 7, which is the study you cite in your report. Can you take a minute to refresh your recollection as to what was going on here. Do you recall the article? 10:36AM
A Yes, I do.
Q Now, as I understand it, what happened here was there was an outbreak in a town which drew its water supply from a water tower and birds had got into the water tower and were pooping directly into the water; does that sound right? 10:37AM
A Let me read on real quickly.
Q Sure.
A Okay. I'm ready. 10:38AM
Q So I'm looking at Page 582 in the right-hand column. Does that basically say what I just said it said?
A Could you repeat what you said it said?
Q Sure. That the source of this outbreak was from the public water tower and birds had infiltrated that and were pooping directly into the water. 10:38AM
A That was the conclusion of the authors, yes.
Q And the water system here was going directly 10:38AM

from the -- give me just a second. The water here was going directly from the water tower to homes without being chlorinated.

A Correct.

Q Do you recall that? Do you -- what are the differences that you would identify between this outbreak, water tower Salmonella deposited into that water sent into people's homes where it's consumed as drinking water; what are the differences between that and the type of exposure we're talking about in the Illinois River watershed? 10:39AM

A Well, again, the question is awfully broad. Can you narrow it down for me?

Q Sure. In this study people in this town were infected after using the Salmonella-infected water as drinking water; correct? 10:39AM

A Correct.

Q So taking cups to the faucet and filling them up and drinking it?

A Correct. 10:39AM

Q Does that kind of activity happen in the IRW?

A People could be ingesting the water through playing, swimming, canoeing but they --

Q Do you think it's likely that people are dipping a cup into the river and taking a chug? 10:40AM

A I would not think that was likely.
Q The second study that you cite here by O'Reilly, et al -- let me hand you this one. Now, you characterize this in your report as a Salmonella outbreak; is that right? 10:40AM
MR. PAGE: What is this exhibit; is this No. 8, please?
COURT REPORTER: Yes.
A Yes, I did, uh-huh. It actually included Campylobacter and Salmonella. 10:41AM
Q Right.
A So multiple etiological agents.
Q Okay. If you look at the summary of this study on the first page, after results, the first sentence there, would you read that for me? 10:41AM
A That starts with conclusions?
Q No. Above that that starts the results among.
A Among the 1,450 persons reporting illness, Campylobacter jejuni, norovirus, Giardia intestinalis and Salmonella enterica serotype 10:41AM Typhimurium were identified in sixteen, nine, three and one persons respectively.
Q Do you think it's fair to characterize this as Salmonella outbreak if Salmonella was identified in one person? 10:41AM

A Well, if you notice that there were -- out of the total people getting sick, 1,450, the etiological agent, the organism was not identified in very many of them. So they were obviously having a lot of trouble in determining the cause in most of the illness. So, yeah, I mean, I think it's fair to say that Salmonella contributed to the illnesses that were here since it was identified. 10:42AM

Q Okay, but that's not what you did in your report; you characterized this as a Salmonella outbreak. Do you think this is a Salmonella outbreak? 10:42AM

A I think it's an outbreak that involved Salmonella certainly. It also -- sorry.

Q But it's not a Salmonella outbreak? 10:42AM

MR. PAGE: Object to the form.

A It also involved some other etiological agents.

Q Just trying to understand. Let's move on to pathogenic E. coli. Have you ever studied pathogenic E. coli as an organism? 10:42AM

A Can I go back to that for just one second?

Q Sure.

A Because in my report it does say -- so to quote, in 2004 an Ohio town was the site of an 10:42AM

outbreak caused by contaminated drinking water that included Salmonellosis and Campylobacteriosis. So I think that what I said in my report was quite accurate actually.

Q So you are not characterizing this as a Salmonella outbreak? 10:43AM

A It's an outbreak that involved Salmonella, in which Salmonella was identified.

Q Okay. Moving on, have you ever studied pathogenic E. coli as an organism? 10:43AM

A I don't think I've ever done a study that directly tested for pathogenic E. coli.

Q Have you ever studied its infectiousness rate?

A No.

Q Have you ever published anything about it? 10:43AM

A Not directly, not a whole paper about pathogenic E. coli.

Q And I take it the plaintiffs have not done any testing for pathogenic E. coli?

A That's correct. 10:43AM

Q You note in your report drug resistant E. coli. What type of E. coli are these?

A In general the drug resistant E. coli that people are investigating are the non-pathogenic type. So the danger of these organisms is they will 10:44AM

spread their antibiotic resistant genes to pathogens and then make the pathogens more difficult to treat.

Q Is there any literature about drug resistant pathogenic E. coli?

A Yes, definitely. 10:44AM

Q Do you have any -- have you done any study of drug resistant pathogenic E. coli in the IRW?

A No.

Q One of the -- let me give you a source on this. This is an article by Leclerc, et al, this 10:44AM was among your considered materials, and again I didn't copy the whole article for you. I just copied the relevant portion to save some paper. If you turn to Page 375 --

A All right. 10:45AM

Q -- the bottom right about six lines up, it says VTEC, including E. coli 0157:H7 are strongly associated with cattle and they can clearly pass through the stomachs of ruminants. Do you agree that pathogenic E. coli are most strongly associated 10:45AM with cattle?

MR. PAGE: Object to the form.

A Yes, I agree that they're most strongly associated with cattle, but they are found in poultry. 10:45AM

Q What about -- out of curiosity, what about the second part of that sentence that says they can clearly pass through the stomachs of ruminants; do they pass through of animals' digestive systems intact sometimes? 10:45AM

A Yes, they can sometimes pass through.

Q And be excreted in a viable form?

A That would happen I mean relative infrequently. You'd more often expect to find bacteria that live in the intestines. The 10:46AM pass-through organisms would tend to die off, but I suppose it's possible.

Q Crops sometimes can take bacteria up, actually up inside them, right, through their roots, through the irrigation process, so the bacteria is not just 10:46AM on the surface but is actually internal to the crop. Are you familiar with that?

A I haven't heard of that phenomenon, no.

Q Okay. Let me ask you I guess one final question on that. You've made no study of whether 10:46AM the Brevibacteria that you identified through your PCR process could pass through an animal?

A We have not studied that.

Q The next chunk of your report deals with water quality testing and I think we pretty well beat the 10:46AM

water quality indicators to death the last two times we've spoken to you, so I'll just be very brief about it. At the PI hearing, the preliminary injunction hearing, you testified that in your view the EPA has committed to maintaining the use of 10:47AM
Enterococci as an indicator of risk to human health in fresh water -- in recreational fresh waters. Do you recall that?
A I recall talking about Enterococci and the fact that the EPA intends to continue their use in 10:47AM
the near future.
Q And do you recall being asked about the Wade Meador review from 2007?
A I recall -- I don't know what I was asked about it but I recall talking about it. 10:47AM
Q Okay. Do you remember that discussion -- this is just background -- that discussion had to do with the statement in that Meador review that based on the studies it looked at, that only E. coli was clearly associated with an increase in the 10:47AM
relatively risk of disease and is, therefore, a more reliable indicator than Enterococci; do you remember Mr. Jorgensen presenting you with that quote?
A Yeah, we talked about that, and there was very few studies with Enterococci, which was a problem in 10:48AM

that meta-analysis.
Q Okay, and that's what I wanted to ask you about. Your testimony then was that more recent studies have demonstrated that Enterococci is associated with health risk in recreational fresh waters, and I was wondering if you can identify those for me. 10:48AM
A Do I have them in my report?
Q Well, in Paragraph 30, you cite Dr. Teaf for the possibility -- for the proposition that Enterococci are responsible for many of the water quality exceedances throughout the IRW. 10:48AM
A Right, but about the -- the more recent EPA studies would include -- I'm drawing a blank on the guy's name. There was one by Wade or that Wade was a co-author on, and his name starts with an H, but at any rate, yes, there are more recent studies that have shown this correlation and specifically with qPCR, quantitative PCR for the Enterococci. 10:48AM
Q These are studies using qPCR? 10:49AM
A Yes.
Q Okay. Can I push you a little harder to remember anything you can about these studies because this is pretty important? At the hearing you asserted their existence but didn't name them, 10:49AM

so I would like to know what they are.
A Haglund, Rich Haglund, H-A-G-L-U-N-D I believe.
Q Okay. Any others?
A I think Haglund and Wade were on both of the studies, two conducted by the EPA. 10:49AM
Q Okay. Whatever these studies are, would you have looked at them in preparing your report?
A I probably looked in them in doing other things, and so that's why I remembered them, but I didn't specifically refer to them in preparing this report. 10:50AM
Q Do you think they would be in your considered materials?
A It might -- I don't know. I'd have to check. 10:50AM
Q Okay. One other question on the indicator bacteria. Is it your view that the source of fecal indicator bacteria at a particular location, i.e., whether it's human or animal, is it your view that that's irrelevant to the utility of the indicator bacteria as a prediction of risk to human health? 10:50AM
A No, it's not my view that it's irrelevant. It's my view that one needs to know the source of the indicator bacteria in order to begin to conduct an accurate assessment of risk. 10:50AM

Q How does knowing the source help you conduct an accurate assessment of risk?
A Knowing the source helps you know what pathogens are likely to be associated with that on fecal contamination. 10:51AM
Q Now, you note -- we're on Page 10 now. In Paragraph 24, you note that water quality rules provide both single sample and geomean standards, and under Oklahoma -- am I correct that under the Oklahoma rules a geomean is based on at least five 10:51AM samples in a period of no greater than 30 days?
A Those -- the geomean requirements are actually for establishing regulatory rules, but that's correct, for establishing the regulatory rules.
Q Okay. What is the purpose of the 30-day 10:51AM period?
A The purpose of the 30-day period is to reflect a relatively short time frame over which the samples were collected.
Q Why does that matter? 10:52AM
A It's a little bit of a historical anomaly I think but -- and the regulatory agencies rely -- it's a historical anomaly in that the bacterial standards are actually based or vary depending on whether one would use the water frequently or 10:52AM

infrequently, and so the geomean is intended to reflect frequent -- the geomean is intended to reflect frequent use of the water over some defined time period, and so that's why they constrained those five samples into 30 days. 10:52AM

Q How broad a period -- you say it's a historical anomaly, so I take it you disagree with the 30-day limit?

A I think it's very overly restrictive.

Q How broad a period of time do you think it's 10:53AM appropriate to use samples to characterize the bacterial health of a river?

A When I do studies, I prefer to get a broader snapshot, so, say -- I mean one year is really to me a snapshot of water quality in a river, and then if 10:53AM you can have a two or three-year time period, then that's even better.

Q Okay.

A So covering seasons I think is really important, and that 30-day geomean definitely 10:53AM doesn't cover seasonal variation.

Q I take it then that this is the bacterial -- I'm not sure what the appropriate term is to phrase this question. Does the bacterial makeup of a river vary with seasons? 10:53AM

MR. PAGE: Object to the form.
A Can you clarify what you mean by bacterial makeup?
Q Sure. That's what I was struggling to find the correct technical to talk to you. The types of bacteria that are in a river, do they very seasonally? 10:54AM
MR. PAGE: Object to the form.
A So it would depend on what type of -- it would depend on a lot of factors in the river as to whether, but you could have -- well have seasonal variation depending on factors, like rainfall, for example, would be a major one in Florida. 10:54AM
Q Okay. Are there any other factors that would affect differences in bacterial makeup of a generic river from season to season? 10:54AM
A If you had a river that had a lot of trees over it and then shed their leaves so you went from being a very shaded river to an open river, then that could, for example, cause the microbial concentrations that were in the river to vary, so you have water influencing what went in and then the amount of shade influencing what survived once it got in there. 10:54AM
Q Okay. You said one year would give you a 10:55AM

snapshot. Two to three years would be even better in your mind. How many samples -- to really understand what's going on bacteriologically in a river, how many samples would you want over, say, two years? 10:55AM

MR. PAGE: Object to the form.

A I think that's really hard to determine. It would depend on the size of the river, the amount of seasonal variability that you have, the amount of other intrinsic variables you have, in other words, 10:55AM you know, are animals there sometimes and other times not, certain animals. So it really would vary. There's some rivers in Florida where we do studies based on quarterly sampling, others where we sample monthly and, again, it would be different 10:55AM depending on the river.

Q Does it depend in part on the question you are trying to answer?

A Yes, it would depend in part on that.

Q Okay. When you take, say, the Illinois River 10:56AM watershed, for instance, people recreate in the river for a limited period of time each year; correct?

A Correct.

Q And so the regulatory interest is in knowing 10:56AM

how healthy the water is at the time that people are recreating; that is correct?

A Well, no, because TMDLs are not only about people recreating. Total maximum daily load regulations are not only about people recreating, they're actually about the condition of the river overall and that applies to nutrients and its effect on the flora and fauna. So, no, you wouldn't just be interested in when people were in the water. 10:56AM

Q Okay. If I wanted to know the degree of health risk in, say, July of a given year, would I be able to know that accurately from five samples taken over the preceding three years? 10:56AM

MR. PAGE: Object to the form.

Q Distributed evenly over the preceding three years? 10:56AM

MR. PAGE: Same objection.

A I can't really answer that without knowing more about the water in question and the types of -- the type of water body in question and, again, how much -- how many extrinsic factors were you influencing. 10:57AM

Q You can't answer that, so I take it there would be some set of circumstances where you think you could know that, you could know the answer based 10:57AM

on those five samples?

MR. PAGE: Object to the form.

A I -- you could have information about it. I'll put it that way. I don't know how complete -- I can't say how complete that information would be. 10:57AM

Q Okay. You refer in Paragraph 32 on Page 13 -- let me see where this is. Fifth line up from the bottom you refer to exceedances as being chronic. When does an exceedance become chronic; what's your use of that term? 10:58AM

A I can't put a number to that, but I would say chronic would be when you note them, A, through the watershed and, B, over a period of time and not confined to one particular time of year but reoccurring. 10:58AM

Q Have you calculated for any particular segment in the IRW how often the running 30-day geomean standard exceeded applicable water quality standards?

A I have not. 10:58AM

Q Do you know whether the State has sufficient data to calculate a 30-day geomean for any segment in the IRW?

A I don't know that for sure. I do know that about 75 percent of these waters are impaired. 10:59AM

Q Do you know whether the exceedances are predominantly single sample or geomean?
A I don't know that off the top of my head.
Q How common is a single sample exceedance after several days of no rain? 10:59AM
MR. PAGE: Object to the form.
A Do you -- can you clarify? Do you mean in the IRW or in general?
Q In general. You've studied rivers, so a hypothetical river, would you expect to get a single 10:59AM sample exceedance after, say, a week of no rain?
A That's really highly dependent on the water body. So that's definitely something I can't answer.
Q What type of factors would you need to know? 10:59AM
MR. PAGE: Object to the form.
A Well, okay. Can you clarify the question, so --
Q Well, I asked you whether a single sample exceedance after a week of no rain is common, and 11:00AM you said it would depend on the water body. So I assume there was some things you have to know. I had would like to know what they are.
A There are some things that I would have to know in order to do what? Like I would go out and 11:00AM

sample the water body and see --
Q Okay.
A -- whether it exceeded or not.
Q Okay. Well, you said it would depend on --
we're trying to build a hypothetical here, a hypothetical river. Let's put that aside. Okay. Let's say in the IRW, on, say, the main stem of the Illinois River watershed, would you expect to find a single sample of bacterial -- or a single sample exceedance after a week of no rain? 11:00AM
A Now that I cannot answer because I have not seen or at least I haven't analyzed rainfall data. I don't think I've seen rainfall data for the IRW. So I don't have a feel for whether the exceedances in the IRW are dependent on rainfall or not. 11:01AM
Q Okay. What else could they be; if not rainfall, what could be the other sources of bacteria?
A I'm really not following this hypothetical. I'm sorry. 11:01AM
Q Well, when it rains, rain washes bacteria from surfaces into surface water; is that correct?
A That's correct.
Q So that is one -- overland flow is one source of bacteria? 11:01AM

A Correct.
Q If we take that source out of the picture and so there's no rainfall that's carrying surface bacteria into surface waters, where else could bacteria come from to lead to a single sample exceedance? 11:01AM
A They could be in the sediment, so then if people, for example, are floating on the river, then they could stir them up from the sediments.
Q Anywhere else? 11:02AM
A Again, that would depend on whether the river was just at -- had chronically -- so the river, if it had chronically high level, then you know there's inputs coming from somewhere, but if the levels spike up and down, then that's going to be a different phenomenon, but the factors that influence the water quality in these places are so complex that I'm just having a really hard time. 11:02AM
Q What carries Brevibacterium; the Brevibacterium identified by the PCR process, what carries it to surface water in the IRW? 11:02AM
A It would be brief -- there could be some airborne contribution when the litter is spread, but I would hypothesize that most of it is coming from water flow. 11:03AM

Q Surface water flow?
A Surface water flow, and then there would also be groundwater contributions from whatever has gotten in through the Karst and percolated through there. 11:03AM
Q Okay, but based on the sampling data you've seen, the vast majority of it is surface water; is that right?
A The numbers are very -- are the highest in surface water that is coming off in these edge of 11:03AM field samples.
Q Would you expect to find Brevibacteria on the main stem of the Illinois River watershed after a week of no rain?
A Again, I can't -- same hypothetical that we 11:03AM just talked about, there are so many factors that would influence, that I really would have a hard time saying that.
Q What are those factors?
A Again, how -- so how it's getting into the 11:04AM water would be important.
Q I guess that's my question. How is it getting into the water if we take rain out of the picture?
MR. PAGE: Object to the form.
A So you could have -- when you are spreading, 11:04AM

you could have airborne deposition in there. Then the other major thing that I can think of, again, would be if it gets deposited in sediments along with E. coli and Enterococci.

Q Have you done any quantification of airborne? 11:04AM

A No.

Q Okay. Have you -- what sediment measures have you taken?

A We have analyzed sediment from various parameters, but we have not actually analyzed the 11:04AM sediment for the Brevibacteria, for the biomarker.

Q Are there any other -- other than rain, surface water, airborne and sediment, are there any other sources that you want to posit?

A Of Brevibacteria? 11:04AM

Q Sure.

MR. PAGE: Excuse me. Is it Brevibacteria or the marker?

MR. TODD: Good question.

Q The Brevibacteria, the PCR Brevibacteria. 11:05AM

A So the actual DNA sequence?

Q Well, I guess -- well, the bacteria or for that matter, the sequence that comes from the Brevibacteria, would you expect to find either?

A Again, there's just so many -- there's so many 11:05AM

confounding factors that I can't say. I don't know.
Q Can you list some more of the confounding factors?
A Well, I think I went through -- the biggest one would be if the litter is deposited on the field and it's not raining, you know, does it blow off into the water, for example? I mean that's another way that it could be deposited. That would be some of the biggest ones. 11:05AM
Q Okay, and you mentioned sediment. Are there any other confounding factors? 11:05AM
A That's the biggest one is whether it's residing in sediment.
Q So that's two. Are there any others? Your saying it's the biggest one suggests there are others. What are the others? 11:06AM
A Those are the biggest ones I can think of off the top of my head.
Q How quickly does it take bacteria, land applied bacteria in a field in the watershed, how quickly does it take to reach surface water? 11:06AM
A I don't know.
Q Have you ever measured that?
A No.
Q Okay. Do you think it takes days, weeks or 11:06AM

months?

MR. PAGE: Object to the form.

A It would depend on how quickly or how direct the route was. So if it was going to reach the water via percolation into the groundwater and out, then that might take weeks. If it was going to reach it through direct surface runoff, then usually what you see with bacteria is a peak within three to seven days of rainfall. Again, depending on the topology of the watershed and how direct the route of access is. 11:06AM 11:07AM

Q Let's talk a little bit about bacteria found in litter. In Paragraph 31 of your report, and this is on Page 13, you characterize levels of E. coli and Enterococci in the litter samples that the State tested as being extremely high. Do you see that? It's in the middle of the first chunk of the paragraph. 11:07AM

A Okay.

Q Do you see that? 11:07AM

A Uh-huh.

Q What do you mean by extremely high; high as compared to what?

A High would be compared to my knowledge of surface waters that were not unimpacted but that 11:08AM

were relatively clean.
Q Okay, but we're talking about the litter samples here; right?
A Oh, I'm sorry, I'm sorry. I was down on environmental samples. Okay. Let me back up here. 11:08AM
We're on Page 13.
Q Page 13, right in the middle, right here.
A Okay. Got it.
Q So extremely high as compared to what?
A I really wouldn't compare it to anything 11:08AM
because if you've got, you know, 1,200 per gram of litter, it's like, you know, the size of a ball bearing or something like that, and that's just a priority extremely high to me.
Q Okay. Is that what you would expect for 11:09AM
material that's been -- that's had feces directly deposited into it?
A That's another really broad question. I mean it would depend on the type of feces and where the feces was and all those sort of things. 11:09AM
Q Okay. Well, you used the term extremely high in your report, which is a relative term. So I'm wondering what is your baseline; what baseline should the court draw, should the jury draw when you say something is extremely high? 11:09AM

A I think, again, that's just -- just looking at that, if you've got over a thousand on something that's -- on a piece of material that that's small, I would consider that extremely high. You extrapolate, you know, that pea size or that ball bearing size chunk or whatever it is out to what would be in a hundred mils of water, which is the sample size that we take for water quality, then you're talking about, you know, multiplying that by at least a hundred, so then you are getting up into the 120,000 if you are, again, extrapolating this bigger size. 11:09AM 11:10AM

Q Okay. So it's just extremely high by any measure?

A It's extremely high, yes. 11:10AM

Q Okay. You note in that same paragraph I think that you note exceedances at certain put-in spots along the river. I'm sorry. It's actually at the next paragraph. It's the second to the last line on the page. 11:10AM

A Okay.

Q Are you talking about specific put-in spots?

A Yes.

Q Okay. Can you identify them for me?

A Yes. They would be in my figure -- maybe this 11:10AM

is not a figure I input. This may be a figure in Dr. Teaf's report that I was thinking that I had in here. Sorry. That figure shows the exceedances at various put-in spots. Sorry about that.

Q No need to apologize to me, and I take it none of those exceedances are calculated using the 30-day regulatory geometric mean? 11:11AM

A The samples were not collected to the best of my knowledge using that 30-day.

Q Okay, and so you haven't looked to see how the regulatory standards are exceeded in these particular spots? 11:11AM

MR. PAGE: Object to the form.

A I -- so I relied on Dr. Teaf's work there.

Q So you yourself have not calculated whether regulatory levels are exceeded or whether the 30-day regulatory level was exceeded at any of these particular spots at any particular time? 11:11AM

A Again, I relied on Dr. Teaf's report.

Q Let me check off a few things here quickly. You mentioned Karst substratum in here. Have you yourself studied Karst substructures in the IRW? 11:12AM

A No, I have not.

Q Okay. You note that various indicator bacteria were isolated from springs, shallow wells 11:12AM

and deep wells. Do you have any personal knowledge of any of these?

MR. PAGE: Object to the form.

A The only personal knowledge I have was in our tour of the watershed, we did stop by some springs and I saw them. That's the only personal knowledge I have. 11:12AM

Q Okay. You pay on Page 14 in Paragraph 33 that owners of wells typically don't disinfect or treat their well water. What is your basis for that statement? 11:12AM

A My experience in the water quality industry.

Q Okay. So that's not based on any particular study of well water owners in the IRW?

A No. It is based on some of the other expert reports that the -- statements in an expert report that that is not a practice in the IRW. 11:12AM

Q Okay, but apart from that other expert's report, that's your basis?

A Correct. 11:13AM

Q And did you rely on Dr. Fisher for the calculation of the number of birds and poultry houses in the IRW?

A Yes.

Q You've also mentioned Dr. Olsen's work. Did 11:13AM

you contribute in any way to Dr. Olsen's PCA analysis?

A No.

Q So when you mentioned his report in your report, you are just relying on his report? 11:13AM

A Correct.

Q This is actually a great stopping point. So why don't we go ahead and do that.

VIDEOGRAPHER: We're now off the Record. We're now at 11:13 a.m. 11:13AM

(Following a short recess at 11:13 a.m., proceedings continued on the Record at 11:29 a.m.)

VIDEOGRAPHER: We are back on the Record. The time is 11:29 a.m. 11:29AM

Q Professor, let's talk a little bit about PCR now. When PCR is used in a hospital, how is it used?

A PCR can be used in a number of ways. One of the most important ways is for diagnosis of disease 11:29AM and confirmation of diagnosis so that a particular gene or genes will be targeted by PCR, and the microbiologist will use that, again, either to confirm or diagnose the presence of a particular causative agent of the disease. 11:30AM

Q So in this instance, would, say, a doctor have a theory as to what disease a patient has and then PCR will be used to confirm that diagnosis; is that what you are telling me?

A The doctor would either be relying on symptoms of the patient or could be relying on isolation and culture of a particular bacteria and then confirmation of its identity. 11:30AM

Q And is the utility of PCR in that setting that it can multiply the DNA; if you have, let's say, relatively a few, it multiplies the DNA to a point that it can be identified? 11:30AM

A The utility of the PCR is partly what you said and increasing the sensitivity, the ability to detect low levels of DNA, but it's also in the specificity of PCR and so that you can use the PCR to definitively identify the pathogen. 11:30AM

Q That is one pathogen as opposed to another?

A Correct.

Q Okay. Same question for when PCR is used in the forensic context; how is it used there? 11:31AM

A In the forensic context, it's generally used as in matching a particular DNA sequence from an individual to DNA that was retrieved from a crime scene, for example. It could also be used in a 11:31AM

paternity type of setting, again, where matching a particular DNA fingerprint of one person to that of another.

Q After you were -- when you were retained to work in this case and you started to work on this case, did you consider microbial source tracking methods other than PCR? 11:31AM

A Not to the best of my recollection.

Q You don't consider or recall considering using antibiotic assistance analysis? 11:31AM

A I don't recall that.

Q Okay. How many published studies have used the same methodology that you have used in this case to create a host-specific assay for fecal pollution?

A The general methodology, that of using a library-independent PCR method, has been used in at least 30 or 40 published studies, but as far as -- so at least 30 or 40. 11:32AM

Q Are you including in those 30 to 40 studies -- are you including in that studies that have used boxed PCR? 11:32AM

A I would be talking about library-independent methods, and so in general, those would not use boxed PCR.

Q Okay. That's the library-dependent method? 11:32AM

A Library-dependent method would be boxed PCR.
Q What about rep-PCR; is that also library dependant?
A That's also library dependent.
Q Okay. So you think there are 30 to 40 studies using or library-independent studies using PCR? 11:32AM
A Yes.
Q Okay. All designed to create a host specific assay for fecal pollution?
A All designed to detect a host specific signal for fecal pollution. 11:33AM
Q Okay. Specific to a specific class of animal or a specific unique animal, such as chickens?
A Sometimes it would be a class of animals. For example, a well-known one is the ruminant marker for the Bacteroides. Other times it might be for humans, for dogs, so that would be obviously more specific. 11:33AM
Q Okay. Are there any studies, published studies that have used the precise methodology that you and North Wind used in this case, starting with the TRFLP and the PCR and the qPCR? 11:33AM
A There are studies that have followed that whole methodology, yes, of identifying the marker with TRFLP and then developing the PCR assay from 11:33AM

that and then following on to develop the qPCR from that.

Q Can you identify -- I'm sorry. Finished?

A Yeah.

Q Can you identify those studies for me? 11:34AM

A The one that I'm thinking of that I'm most familiar with would be Kate Field and I believe Oren Shanks was also on that work, Katherine Field and Oren Shank. Oren is with the EPA now, but I think at the time he was working with Kate Field. 11:34AM

Q Can you identify any others for me?

A Again, that's the one that comes to my mind.

Q Okay. So, no, you cannot identify any others?

MR. PAGE: Object to the form.

A Not off the top of my head. 11:34AM

Q Are there others that you just simply are not recalling?

A I'm not sure. Yes, there is at least one other.

Q Please. 11:34AM

A Seurink, S-E-U-R-I-N-K.

Q And what was that designed to detect?

A Similar function of identifying specific primers and then going to qPCR.

Q Have the plaintiffs used any other or any PCR 11:35AM

assay to detect fecal pollution from any animal other than -- or any creatures other than poultry in the watershed?

A No, no.

Q Okay. At your last deposition we talked about the report that North Wind had sent you which set out the process that North Wind had created to set out the process you used to develop the assay, and that was dated December, and the considered materials that were produced this time around had that December report in them. Has there been -- is there a more recent version of that report? 11:35AM

A That report was the report of the procedure used to develop the qPCR, and there has not been a more recent version of that particular report. 11:36AM

Q There have been more recent data reports; right?

A Yes, that's correct.

Q Okay. Did you ever test -- have you ever tested poultry feces to determine whether they contain the PCR Brevibacterium? 11:36AM

MR. PAGE: Object to the form.

A We have tested contaminated litter to determine that it can contain --

Q Did you ever test poultry feces? 11:36AM

A Directly did we -- no, we have not directly tested poultry feces.
Q And we discussed earlier that you did test some samples of clean bedding material?
A Correct. 11:36AM
Q And you did that to determine whether the Brevibacterium was there?
A To ensure that the marker was not present.
Q Okay. What bedding material did you use?
A I would have to check on that. I know that 11:36AM some of the rice hull material was used, and I just can't recall if all of the samples were the same material or if there was different material used.
Q Do you know who got it, who secured it?
A I don't know. I assume it would be CDM but I 11:37AM don't know for sure.
Q Do you know where it came from?
A No.
Q Was the bedding material enriched before it was tested? 11:37AM
A What would enriched mean?
Q Were any nutrients or anything else added to it to grow bacteria that may be present at low levels before it was tested?
A No. 11:37AM

Q Okay. In poultry feces do you think it's likely that the level of PCR Brevibacterium exceeds the level of indicator bacteria?

A Can you state that question again?

Q Sure. In poultry feces, do you think it's likely that the level of PCR Brevibacterium, the Brevibacterium that you have identified through your process, do you think it's likely that it exceeds the level of indicator bacteria, E. coli, Enterococci, that are contained in the feces? 11:38AM

A That's super hard to say because for the Enterococcus and E. coli, we use culturable methods, so we're certainly underestimating the total DNA numbers; whereas, for the Brevi, we're using, of course, the PCR method. So it's really comparing apples to oranges. 11:38AM

Q Okay. How much do you think culture methods underestimate levels of indicator bacteria?

A In my experience, in stressful situations, like what we have now, up to a hundred to a thousand fold. 11:38AM

Q Okay. Do you think that -- would the relationship between Brevibacterium and indicator bacteria in feces be consistent -- I'm sorry -- be proportional? 11:39AM

A Can you clarify that for me?
Q Sure. Do you think that that would be -- that in poultry feces there would be a consistent proportional relationship between the level of Brevibacterium and the level of indicator bacteria? 11:39AM
A That would certainly depend on the conditions of the litter, for example, how long since the litter had been exposed to poultry, for example.
Q I'm not asking in litter. I'm asking about in feces. 11:39AM
A Oh, I'm sorry. Well, so in feces, would there be a consistent proportional level of the indicator bacteria compared to the Brevibacterium biomarker?
Q Uh-huh.
A I would hypothesize that there would be. 11:40AM
Q Would you expect the Brevi to be dominant or the indicators to be dominant?
A Based on the data that we have now, I would expect that the Brevi might exceed the indicators but, again, that's a hypothesis. It's not something 11:40AM that I've tested.
Q Okay, but based on the data that you have now, what do you expect that relationship to be -- what would you be expect the relationship to be, one to one; order of magnitude, what would you expect it to 11:40AM

be?
A I'm having a real hard time giving you a good estimate because, again, we're working with poultry litter, which is -- we worked with it on purpose because we know that's what is going to be spread on the field and we know that's where we really need to be able to detect it, but we have not assessed the enumerated in feces and so, again, I'm having a difficult time giving you a proportion between because I just don't have that data. 11:41AM
Q Okay. No. I'm just interested in your educated guess there. We distinguished earlier between the Brevibacterium and the actual marker itself, the genetic sequence.
A Correct. 11:41AM
Q In looking for the marker, looking for the sequence, you targeted the 16S gene?
A That's correct.
Q Do bacteria contain more than one copy of the same gene? 11:41AM
A You'll have to clarify that because there's a lot of genes in bacteria.
Q Okay. Are you familiar with any studies where particular bacteria were demonstrated to carry more than one copy of the 16S gene? 11:42AM

A Oh, yes.
Q Okay. Do you know whether Brevibacteria has been studied or have been reported to contain more than one copy of the 16S gene?
A Brevibacterium is -- it's unknown now. In some Brevibacterium species there have been four copies reported. It may have four. Most bacteria do carry more than one copy of the gene. 11:42AM
Q Okay. So when we're looking at the numbers of gene copies for the sequence, we are to divide by, say, four or whatever the number of copies are to get to the number of actual bacteria? 11:42AM
A Correct.
Q Okay. How could you -- in order to tell for sure how many copies of this Brevibacteria carries, you have to culture it; is that correct? 11:42AM
A Yes, you would have to culture it.
Q Okay. Now, in the litter -- if you look back at Paragraph 31 of your report, it's on Page 13, it's where we were before, and in the litter -- in the middle of that paragraph, you report finding a geometric mean of 1,200 E. coli per gram of litter and 5,100 Enterococci per gram of litter; do you see that? 11:43AM
A Correct, yes, I do. 11:43AM

Q So that's the geomean of the samples of litter that the State took?
A The concentration of the indicator organisms in the litter, yes.
Q Okay. Now, do you recall the concentration of the gene copies of Brevibacterium that you found in your litter samples? 11:43AM
A I believe it was 10 to the 7th and 10 to the 9th.
Q Okay, and so dividing that these readings by four, do you have an estimated range of number of bacteria in the litter? 11:43AM
A So then you are looking at something times 10 to the 6th to something times 10 to the 8th.
Q I just wrote down the largest litter reading and the smallest litter reading. So let me just -- I'll give you the numbers and then we can look them up if you want to be -- I'll state these for the Record and if they're wrong, we can check it later. 11:44AM
The largest litter reading which was sampled FAC-07-8-3-06, had 2.49E plus 09, so it's to the 9th? 11:44AM
A Yeah, to the 9th.
Q Right, and then there's -- well, side question. When this data is reported, there's a 11:44AM

plus or minus that follows it?

MR. PAGE: Object to the form.

A Right.

MR. PAGE: Object to the form.

Q Is it an error rate or standard deviation? 11:44AM

A It's a standard deviation for multiple samples.

Q Okay. Putting that aside, the number I just gave you translates into -- I don't know what it translates into in words. 11:45AM

A Billions.

MR. PAGE: Object to the form.

Q Okay. Billions of gene copies, and if you divide by -- at any rate, it comes out to a number that's many orders of magnitude greater than the 11:45AM number of Enterococci and E. coli that you identified in the litter; correct?

A If you divide those numbers, yes.

Q Okay, and that -- those ratios strike you as reasonable? 11:45AM

A Yeah.

Q Do you think that it's likely that if there is a bacterium that no one has ever cultured previously or identified or that is associated with poultry, do you think that it out -- that in poultry feces or in 11:46AM

poultry litter would outnumber the indicator bacteria by many orders of magnitude?

A So are you talking about Brevibacterium avium there?

Q Well, the Brevibacterium that you identified in the litter. 11:46AM

A Brevibacterium avium has been cultured from poultry.

Q Are you now saying that Brevibacteria that you identified in the litter is Brevibacterium avium? 11:46AM

A It's in distinguishable from Brevibacterium avium based on the DNA sequence.

Q I thought you testified it was 98 percent consistent?

A That's right, and that's indistinguishable. 11:46AM The general rule in molecular biology is 95 to 97 percent identity. Greater than that is the same species.

Q Brevibacterium avium has been isolated in bubble foot lesions on poultry feet; correct? 11:46AM

A Correct.

Q It's not been identified in poultry feces?

A Correct. There's very little out on the organism.

Q Is there any possibility that Brevibacteria is 11:47AM

growing in the litter?
A Is there any -- yes, there's a possibility, but that wouldn't matter for its purpose as a marker.
Q Are indicator bacteria growing in the litter? 11:47AM
A They could be.
Q They could be?
A Uh-huh.
Q What would you look at to determine whether they're growing in the litter? 11:47AM
A You have to do studies. I mean you look at pH; you look at water content. Salmonella, for example, have been demonstrated to increase up to two logs, and litter when the pH and the water content are right, so you could have some growth of 11:47AM pathogens and of indicators.
Q If Brevibacterium were growing in the litter but indicator bacteria are dying in the litter, what would that do to your correlation?
A Well, you could go every single way with that 11:47AM comparison, and you could say this goes up and that goes down, and that goes down and that goes up, and they both go up, they both go down. So it's pretty obvious that if they go different ways, then they're going to be less correlated. If they go the same 11:48AM

way, they stay correlated, but we just don't know. We do know, however, that the numbers are correlated, especially the numbers in the Enterococci, compared to the concentrations of the poultry litter biomarker. 11:48AM

Q We'll talk about the correlations later.

A Okay.

Q You've validated -- you validated the specificity of your assay with non-target fecal samples. Who determined what animals would be used? 11:48AM

A What species of animals?

Q Right.

A That was done in -- that was a collaboration between myself and CDM. I had the most input into it certainly. 11:49AM

Q Okay. Who determined how many samples to collect from each animal?

A Again, that was a collaboration between Roger Olsen and I and -- Roger Olsen and I really.

Q Okay. What factors did you depend on in your recommendation as to collect -- as to how many samples to collect for each animal? 11:49AM

A Really I depended on my knowledge, expert knowledge of being involved in many source tracking studies, and in testing and validating these, these 11:49AM

assays, I really relied on my experience there.
Q Okay. Did you perform any calculation to ensure that the sample size of feces, fecal samples collected for each animal was representative of the population of the animal in the watershed? 11:49AM
A There are no calculations to do that as far as you know.
Q Who determines the location from which samples would be collected?
A That was -- so the general sampling strategy 11:50AM of collecting some samples in the watershed and outside the watershed was agreed upon by -- between Roger Olsen and I and also talking to North Wind Lab, but the exact venues where the samples were collected was by CDM. 11:50AM
Q Did you take any steps to ensure that the sampling locations were representative of the entire watershed?
A I had assurance that they were collected from throughout the watershed, and then having -- and 11:50AM from separate farms which we agreed upon and then knowing that somewhere inside and outside the watershed there was also an assurance of having distribution of samples.
Q Okay, and that was the extent of the steps to 11:50AM

make sure that they were representative?

MR. PAGE: Object to the form.

A And then knowing that we were collecting composites of fecal samples, so that you're basically not relying on one animal but on the feces of several animals to make up a composite. 11:50AM

Q I'm not talking about number of samples. I'm talking about the locations from which they were collected.

A The location, again, inside and outside the watershed, separate farms was important, and other than that, that was the responsibility of CDM to ensure that. 11:51AM

Q Okay, and you had -- did you have any personal involvement in the collection of samples? 11:51AM

A No.

Q Since your last deposition, what additional fecal samples have been tested?

A Some cattle samples from outside the watershed have been tested, and so I believe it was seven additional cattle samples were tested from different farms. 11:51AM

Q What was the need to test additional samples?

A We tested additional samples because the one contamination event that we had had in cattle feces 11:52AM

previously in the lab was -- made us think, okay, so we'll just go out and get more cattle samples so that we can bolster the specificity of the analysis. That was a contamination event but, of course, it was not interpreted as such by the plaintiff's team. 11:52AM

Q Now, the North Wind report from December noted that the primers that you used actually did reproduce Brevibacteria casei. Am I pronouncing that correctly?

A Casei. 11:52AM

Q Casei?

A Yeah. I'm sorry, I lost you there.

Q The primers that you used reproduced casei?

A The primers that we used -- no, they did not amplify casei. 11:53AM

Q Did not amplify casei?

A No.

Q Sorry. Give me just a minute. This is my copy of that report. I didn't bring it as an exhibit. 11:53AM

A Okay.

Q But you're familiar with that. We'll just represent for the Record that I'm showing you the December North Wind report. You see the highlighted portion there. I read that to say the primers you 11:54AM

were using amplified Brevibacterium casei. Am I wrong about that?

A Oh, yes. I'm sorry, yeah. I misremembered that. So they did amplify Brevibacterium casei, which I completely misremembered that. The casei is very closely related to the avium, and so the way you distinguish them is by the melt curves from the SYBR Green assay. 11:54AM

Q Right, and that was my next question. You used a melt curve to distinguish the two. Explain that process to me. Why is it necessary to -- why is it necessary to use the melt curve to distinguish the two? 11:54AM

A So one of the advantages of that, of the PCR on the SYBR Green chemistry is that you can very specifically and very -- or very precisely raise the temperature in the instrument, in the thermocycler, and at a certain temperature point, that DNA will denature. The double strands will break apart, and the breaking apart of those strands is highly dependent upon the actual sequence of the DNA, and so by using a melt curve, then you can distinguish among PCR products that are the same size but actually have different sequences, and that's also commonly used in medical applications. 11:55AM 11:55AM

Q How accurate is a melt curve in distinguishing closely related sequences; is it 100 percent accurate or not?

A Well, here we have -- I believe the casei and the avium sequences are about 95 percent identical, so it can distinguish between those. 11:56AM

Q Okay, but are there instances where two sequences are so closely related that they produce a melt curve -- melt curves that are indistinguishable from each another? 11:56AM

A Yes, that can happen.

Q Did you check to see whether the primers that you used reproduced any other type of closely related Brevibacterium?

A There's only about five different Brevibacterium species that have been identified. So we did not check any of those others, no. They are further apart in sequence than Brevibacterium casei. So based on their DNA sequence, those primers should not amplify from those. 11:56AM

Q And so did you order Brevibacteria avium itself?

A No. I'm not sure it's available in culture collection.

Q It's not, okay. And so for sure, it follows 11:57AM

that you didn't test to see if your primers would reproduce Brevibacteria avium?

A Right.

Q Okay, and you didn't use a melt curve to see if what you have is distinguishable from Brevibacteria avium; is that right? 11:57AM

A Yes. The melt curves were used on all of our samples and on our clones but not against the cultured avium.

Q What -- stupid question. What equipment do you use to read the melt curve? 11:57AM

A What equipment do you use?

Q Yeah.

A It's included in the software of the thermocycler. 11:57AM

Q Thermocycler?

A Yeah. The thermocycler is the PCR instrument that does the -- that does all of the routine of heating and cooling and --

Q Okay. That's probably the answer to my question then. Is there a margin for error associated with that -- with a thermocycler? 11:58AM

A I'm not sure about that. You'll have to clarify what you mean by margin of error.

Q How specifically can it read one melt curve 11:58AM

versus another?
A My lab didn't do the analysis, so I don't know the increment capability of the North Wind instrument, but many instruments are in increments at 0.1 degree centigrade, but I'm not familiar with the increments off the top of my head for the North Wind instrument. 11:58AM
Q How many environmental samples did you test all totaled for the PCR sequence?
A I believe, not counting the fecal samples, I believe it was 237. My weakness is numbers, so hopefully I'm not wrong. 11:59AM
Q That's okay. It's one of my weaknesses as well. Let's go to Paragraphs 44 and 45 of your report, if you would -- I'm sorry, 54 and 55. This is where you set out the results of the testing, and I'm just a little unclear following your write-up as to how many tests and results you are identifying here, so I wanted to go through it with you and make sure I'm understanding how many. Walk through your Paragraph 54 for me, if you would, and tell me how many soil -- how many samples of each type you are testing and what the results are. 11:59AM 12:00PM
A Okay. So we have 10 litter samples, we have 40 soil samples and we have 187 water samples. 12:00PM

Q Okay. Now, if you move on down, you talk about the concentration of the PCR sequence, where you find it, where it's quantifiable. Can you walk through those numbers for me?

A Starting with 2.2 times 10 to the 7th? 12:01PM

Q Starting with the next sentence, the PLB was high enough.

A Oh, okay. So in terms of being high enough to be quantified by the qPCR, 34 of the water samples it was quantifiable, and that includes the 16 -- so 12:01PM that 34 includes the 16 edge of field samples. It also includes a groundwater sample and a spring sample, which I simply broke out from being groundwater.

Q Okay. 12:01PM

A 6 of the 10 soil samples, so 60 percent were quantifiable with respect to the biomarker, and then this just shows the highest amount that we detected.

Q I'm sorry, 6 of the 40 water -- 6 or the 40 soil samples? 12:01PM

A 6 of the 40 soil samples had quantifiable levels, right.

Q Okay. Now, we go to the next paragraph.

A And all of the litter samples had quantifiable. 12:02PM

Q Right, 10 of 10?
A Yeah.
Q In the next paragraph you talk about samples that were below the detection limit?
A For the qPCR. 12:02PM
Q Right, on the qPCR. So could you walk through the results there?
A Sure. So here -- again, this is taking into account all 40 samples. So I'm including quantifiable in these -- quantifiable and present in 12:02PM these numbers.
Q That's what I assumed. I just wanted to make sure I'm breaking it out as you intended.
A Right. So we have total 40 soil samples, and of those, 38 had detectable levels. So if they had 12:02PM detectable levels -- if they is quantifiable levels, then they also had detectable levels. So 95 percent or whatever that is, 90 some percent of the soil samples had at least detectable levels of the biomarker, and that includes the 6 that had 12:02PM quantifiable levels.
Q Leaving 32 as below detection?
A Uh-huh.
Q No. I'm sorry. As present?
A Leaving, right, 32 present but not enough to 12:03PM

quantify.
Q Okay.
A And then of the -- so we had 187 total water samples. 88 were detectable, so that leaves 99 below the detection limit. 12:03PM
Q All right.
A And then I talk a little bit more about the spring and groundwater samples specifically, but those were included in the total of 187 water samples. 12:03PM
Q Okay, and that's actually the only place where I think I lost you or I was unclear. It's one spring, one surface groundwater and one regular groundwater?
A One spring, one -- yes, uh-huh. 12:03PM
Q Okay, good. Then I got it right. So let me -- I think we'll come back to the chart later. I just wanted to in graphically to make the deposition a little easier to read.
Now, to make sure we're all working from the same dataset, am I correct that North Wind ran these samples and they sent you reports which set out the results -- 12:04PM
A That's correct.
Q -- of that testing? I'll walk through the 12:04PM

pathway here.

MR. PAGE: Mr. Todd, could I just ask a question?

MR. TODD: Sure.

MR. PAGE: At the top of this -- is this Exhibit No. 10? It says Paragraphs 44 and 45. 12:04PM

MR. TODD: You're right. It's --

MR. PAGE: Is it a typographical? For the Record, can you correct that, please?

MR. TODD: Yes, sir. That should be 54 and 55. It's the same mistake I made just now. 12:04PM

MR. PAGE: Thank you.

MR. TODD: Good catch. Thank you.

MS. WARD: Also while we're talking, you guys are starting to talk all over each other, and I'm sure it's really hard for the court reporter. She looks a little bit frustrated. 12:04PM

MR. TODD: She's promised to kick me if I cause her any difficulty. There -- I was just kicked. For the Record, I was just kicked by the court reporter. 12:05PM

Q Let me get this. The handwriting on this is mine for the Record, and to let you know what I'm handing you here is I went through the reports that were included in your produced material -- 12:05PM

A Okay.
Q -- from North Wind, and I matched up the samples that were reported in each of those with the Excel spreadsheet that was included in your material which seemed to compile all of those, those reports, 12:05PM
and your spreadsheet referred to them as Set 1, Set 2, Set 3 and Set 4.
A Okay.
Q So what I've done is I've just pulled -- I left out all of the surplus pages and just had the 12:05PM
actual data reports. So let me just represent for the Record that's what these are.
Professor Harwood, do these look like the reports that you were getting from North Wind reporting data? 12:06PM
A Yes, they do.
Q Professor Harwood, look at -- Exhibit 12 is an Excel spreadsheet that was in your considered materials, and the file name -- I'll put this in for the Record -- was Harwood 00000059 underscore 12:07PM
poultry biomarker qPCR summary data current, with current all caps, dot XLS. Does this spreadsheet look familiar to you?
A Yes, it does.
Q Okay. Is this the spreadsheet on which you 12:07PM

maintained your total tally of data reports that you had from North Wind?

A Actually this spreadsheet was prepared by CDM.

Q Okay. Does this spreadsheet, insofar as you understand it, reflect the total data reports for sample testing for qPCR? 12:07PM

A I think this very well may not have Set 4 on it, but I need to look at it.

Q Well, if you look at the -- I believe if you look at the last page and then come in and if you look in under the set column, I think you'll see Set 4 there. 12:08PM

A Yeah, I do see Set 4 there.

Q Okay. Is this the chart that you relied on in culling out the numbers to include in your report? 12:08PM

A I -- no, I cannot recall exactly how I did those numbers.

Q Okay. This was -- in the Excel file that we received, this was actually Sheet 2 and there was another sheet that was Sheet 1, and I'll hand you that now. 12:08PM

MR. PAGE: I'll object to the form. I'm not sure, Counsel, just what you referred to as this.

MR. TODD: I'm sorry. Exhibit 12 was Sheet 12:09PM

2 on that Excel file and Exhibit 13 was Sheet 1.
Q Does this look familiar to you?
A It looks -- I mean I can't say if it's familiar or not because it's out of context, but I mean it looks like a list of samples. 12:09PM
Q Do you have any recollection of whether this came from CDM along with Exhibit 12?
A No, I don't. I'd have to look at my titles and the date that it was done, so I can't say just getting it this way. 12:10PM
Q Okay. There are -- if you'll accept my representation on this, there are four samples that are on Exhibit 12 that are not on Exhibit 13.
A Okay.
Q And let me get you to flip to the second to the last page of Exhibit 12, the second to the last page. If you look in the sample name column, if you go down six, you'll see the sample called Marth Guinn 72506. 12:10PM
A Okay. 12:10PM
Q Okay. Does that sample mean anything to you?
A No.
Q Okay. If you go on down to about the middle of the page, there's a sample RS-3-01-9-25-06.
A Say it again, RS-3 -- 12:11PM

Q -3-01-9.
A 25-06?
Q That's right.
A Uh-huh, I see that one.
Q You see that one? 12:11PM
A Yeah.
Q Now, if you go down two to RS-340-BIO, do you see that one?
A I see that one.
Q Then if you go down four to RS-43-BIO-8-10-06, 12:11PM do you see that?
A Yes.
Q If you accept my representation that these four samples do not appear on Exhibit 13 --
MR. PAGE: Object to the form. 12:11PM
Q -- and my question to you simply is, is there any reason that you're aware of as to why they would be included on one form and not the other?
A No, no reason that I'm aware of.
Q Attached to -- attached to your report is a 12:11PM list of samples where levels of the PCR sequence were detectable. If you would, flip to that, please. It's Table 5.
A I'm there.
Q Is this -- this should be a comprehensive list 12:12PM

of every sample where quantifiable levels were detected.

A This should be.

Q Did you put Table 5 together?

A Yes. 12:12PM

Q On the first page of Exhibit 12, 16 -- it's 15 -- 15 samples down is a sample named EOF-SPREAD-53E-01-4-29-06.

A I see it.

Q Okay. Can you tell me whether that sample was included on Table 5? 12:13PM

A I can barely read this. I do not see it.

Q Okay. If you would take Exhibit 12, and I apologize for having you do this, but would you please add up the number of samples that are reported as being below the detection limit? 12:14PM

A On all of Exhibit 12?

Q Yep.

A There's no way I'll get this right. I get 104 just counting. 12:16PM

Q That's what I came up with as well. So if this chart accurately tracks the North Wind -- the reports from North Wind, then the number of quantifiable samples should be 51 and the number below the detection samples should be 104? 12:16PM

MR. PAGE: Object to the form.
Q Is that right?
MR. PAGE: Object to the form.
A I'm getting a little lost in the math, but I will certainly go back and I'll have to revisit these because I'm not sure how many samples are on this one. 12:16PM
Q Okay. Do you recall in September of 2007 -- does Exhibit 14 look familiar to you, Professor?
A Yes, it does. 12:17PM
Q Can you tell us what this document shows?
A This is a qPCR analysis result from some litter samples and some water and soil samples --
Q Okay. I can give you the --
A -- collected in the IRW. 12:17PM
Q These are -- if you'll accept my representation, these are the samples that were included in the North Wind -- the December report that we discussed earlier.
MR. PAGE: Object to the form. 12:18PM
Q I can give you the page if you'd like to see it.
A Yes.
Q If you compare Exhibits 14 and 15, I think you'll see that they're the same samples. 12:18PM

A Where is Exhibit 15? Oh, this is --
Q Right. 15 is the chart, Table 9 from the December North Wind report, and 14 is the report of the data results dated September 17th, 2007.
MR. PAGE: Object to the form. 12:19PM
A So that's the December North Wind report and this is the analytical report?
Q Correct.
A I think I'm following you.
Q Do you agree that the analytical report shows the data that was included in the December North Wind report? 12:19PM
MR. PAGE: Object to the form.
A I would have to look back and see what data was included when because we had different datasets coming in and they were in different stages of being completed, but if you were to match up all the samples, I mean so far as I can see, it looks like the same samples are appearing on both documents. 12:19PM
Q Okay. 12:20PM
A But I've only looked at a couple of them.
Q Well, if you want to take a couple of minutes to look at a few more, feel free.
MR. PAGE: Do you have the full North Wind report, Counsel, the December report you are taking 12:20PM

excerpts from?

MR. TODD: Yeah. It's the document I pulled out earlier. Unfortunately I don't have complete copies of it. If you'd like me to put it in the Record, I'm happy to do it. 12:20PM

MR. PAGE: You are talking about Document No. 11, Harwood Exhibit No. 11?

MR. TODD: I'm sorry. I'm talking about Exhibit 11 when?

MR. PAGE: 11 one? 12:20PM

MR. TODD: I'm not sure what you're talking about now.

MR. PAGE: Well, what -- I'm not sure what the hell we're comparing frankly, but I'm just trying to follow. You're trying to have her look at 12:20PM a December report. Have you provided the cover page and Page 24 and 25 of the December North Wind report?

MR. TODD: Correct.

MR. PAGE: And you're saying that's the 12:21PM same report as Exhibit No. 11?

MR. TODD: I'm asking if the samples reflected on Pages 24 and 25 of the December North Wind report are the same samples that were reported to Professor Harwood by North Wind on the report 12:21PM

dated September 9th or I'm sorry, September 17th, 2007.

MR. PAGE: Thank you.

A So are the same samples IDs -- so it looks like these sample IDs match up with these sample IDs. 12:22PM

Q Did you match the measurements?

A No. I didn't look at the measurements.

Q Let me have you do that.

MR. PAGE: I'll object to the form of that question. I don't know what you mean by measurements. 12:22PM

MR. TODD: For the Record, the column labeled PCR poultry specific biomarker, paren, copies/UL water or G soil or G litter, closed paren, on each report. 12:23PM

MR. PAGE: Well, then I might be looking at the wrong report. I'm looking at Exhibit 14.

MR. TODD: I'm sorry, yes. Flip to page -- flip over a couple of pages. 12:23PM

MR. PAGE: You're looking at Page 4 of Exhibit 14?

MR. TODD: There you go.

A Okay.

Q Now that you've had a chance to go through 12:26PM

those, would you agree with me with a few minor exceptions or a handful of exceptions, these appear to be the same results?

A Yes, I would agree.

Q Okay. I apologize for putting you through all that. Is there any reason that you can think of as to why the data reported on these two charts would not have been included in the spreadsheet we looked at earlier, Exhibit 12? 12:26PM

A No, no reason at all. I'm sure it was just an error, especially since one of them is -- looks like an edge of field sample that is quantifiable, so one would have wanted that in one's report. 12:26PM

Q Okay. If some of these samples were retested later, would it be appropriate to report the results of both tests or just one test? 12:27PM

A If -- it would depend on what the results were.

Q Okay.

A So because we have a record of the, you know, of the testing throughout, then -- well, I guess you'll need to give me an example of what you mean. 12:27PM

Q I mean pick any sample here. Let's say it's tested here and you've got a quantifiable result.

A Uh-huh. 12:27PM

Q Then later -- on a later results report from North Wind, the same sample ID appears with different results. Is there any reason why you can think of that that would happen?

A Yeah. So like, as you said, if one had been retested, for example, it looks like this LAL1C was inhibited in one test and then was present when it was retested. 12:27PM

Q Okay. What about one that wasn't inhibited; would you want to base your conclusion on all the tests that were done or only a subset of the tests that were done? 12:28PM

A Well, if it's done in a stepwise manner so that you have a report and you know one was inhibited and then -- 12:28PM

Q One that wasn't inhibited.

A You could put a qualifier by it but, you know, not everybody would do that if they had confidence in the second test and they knew there was an anomaly in the first test. So you could -- as I said, you could put a qualifier by it and put the previous results or you could not. 12:28PM

MR. TODD: Okay. Let's go to lunch.

VIDEOGRAPHER: We are now off the Record. The time is 12:28 p.m. 12:28PM

(Following a lunch recess at 12:28 p.m., proceedings continued on the Record at 1:34 p.m.)

VIDEOGRAPHER: We are back on the Record. The time is 1:34 p.m. 01:35PM

Q Okay. Professor Harwood, welcome back. The qPCR process, as I understand it, depends in part on a standard curve; is that correct?

A Correct.

Q Tell me what is the purpose of a standard curve? 01:35PM

A The standard cover provides the ability to relate the amount of fluorescence that the instrument is detecting to the copy number, the gene copy number of the target. 01:35PM

Q What's the instrument that you use to measure the fluorescence?

A It's the thermocyler. I think it's an IO Chrome something at North Wind.

Q Okay. How is the standard curve developed? 01:35PM

A The standard curve is developed by taking -- so you have a known quantity of the plasmin, and you do dilutions so you know how much DNA -- specific target DNA is in each dilution, and then you run the PCR on each of those dilutions and you compare the 01:36PM

crossing time, which is the amount of time it takes the fluorescent signal to reach over background. You graph the crossing time versus the number of gene copies in your positive control.

Q Okay. Am I correct that a PCR cycle is not 100 percent efficient? 01:36PM

A A PCR cycle is not 100 percent efficient?

Q Let me ask you the question. Is each cycle 100 percent efficient?

A I'm not really sure what question you're asking there. 01:36PM

Q Does one cycle make a 100 percent replication? Let's say if you have 10 to start with and you run one cycle, do you then have 20?

MR. PAGE: Object to the form. 01:36PM

A Again, I'm really not following you. The amplification is logrhythmic, so each time you run, you're duplicating, you're doubling the cycle time or doubling the number of copies.

Q Okay, and so following on that, does each cycle precisely duplicate the number of copies; is it 100 percent duplication or some number less than 100 percent? 01:37PM

A It can be a little bit less than 100 percent.

Q Do you know what the efficiency rate is of the 01:37PM

procedure that North Wind developed?
A Well, the standard curve has a 99.9 percent correlation so it's obviously very efficient, but I don't know what the efficiency is, no.
Q I'm sorry. The standard curve has a 99.9 percent correlation? 01:37PM
A Yeah.
Q To what?
A So the R squared value is with the copy number, the gene copy number compared to the CT value is 99.9 something something. 01:37PM
Q Okay. We noted -- go ahead and pull out Exhibit 12, if you would, which is this spreadsheet. You've got it right there in front of you. If you look at the columns, the column reporting the gene copy numbers and the quantifiable standards for the quantifiable results, and then we noted earlier there's a standard deviation. 01:38PM
A Uh-huh.
Q Can you tell me what that represents, that column represents? 01:38PM
A So the standard deviation represents running three separate samples, and it calculates the amount of variability observed between running those three separate samples. 01:38PM

Q Okay, and that's the deviation from the standard curve?
A That's the -- no. That's the deviation -- that's the variation within those samples.
Q Within just those three samples? 01:38PM
A Uh-huh.
Q Okay. Flip, if you would, to Figure 3 in your report. It's Page 31. This is -- is this the standard curve?
A Yes, it is. 01:39PM
Q Okay, and where it says efficiency 93 percent, what does that mean?
A So that means that basically each replication you're getting 93 percent of the expected amount of fluorescence. 01:39PM
Q The expected amount of fluorescence, okay, so that doesn't translate into gene copies?
A Correct. Well, eventually it would translate into gene copies if you went back to the standard curve. 01:39PM
Q Okay. I'm sorry. I keep flipping between exhibits on you. If you go back to 12 again, if you look on the first page 14 down, it's actually immediately above the one I pointed out to you before, you see it's sample labored 01:40PM

EOF-SPREAD-17A-01-51-06?
A 17 --
Q EOF-SPREAD-17A. It's immediately above the one you put a dot next to before.
A Okay. Got it.
Q If you track all the way across, in the last two columns you see there's a yes, yes. The last two columns both say yes.
A Uh-huh.
Q Okay. Can you tell me the significance of those two columns? 01:40PM
A Yeah. So the biomarker melt peak means that there was a peak obtained at the correct melting temperature, and then other melt peaks observed, that's when we do get a result that has more than one peak in it. 01:40PM
Q Okay. So does that mean that the sample contained more than one sequence?
A Yes.
Q So does that mean the primer is replicated, something else in the sample? 01:41PM
A That means that the primers produced two different products that are discriminated by the melt curve.
Q Okay. Did you do anything to quantify the 01:41PM

level of -- the amount of DNA attributable to these two different sequences?

A No, I don't think that was done in this sample.

Q Okay. So then am I correct that the gene copy number and here, which is 2.48 to the 6th, represents the total of both sequences? 01:41PM

A I believe it would. I'd have to ask Tamzen to make sure or ask North Wind to make sure, but I believe that would include both. 01:41PM

Q Okay. Is there an error rate associated with the qPCR process?

A There is -- there -- so there's variability in -- as always in all scientific methods, there's some availability. As far as error rate, I don't know how to codify that. 01:42PM

Q Did you make any effort to calculate an overall error rate for this process?

A For example, the 93 percent efficiency, so that's showing that the reaction is not 100 percent efficient in amplification. The standard curve being 99 or point -- R square of .999 shows it's very linear and very quantitative, so that's part of calculating the error rate. So the error rates that we measured are low. 01:42PM 01:42PM

Q Okay. What were your criteria for determining the threshold value was within the exponential phase of the qPCR reaction?
A Well, we would have to go back to North Wind for that. 01:42PM
Q That's something you don't know?
A That's something that I wasn't involved in.
Q Okay. Do you recall ever asking for that?
A No.
Q Do you know what the controls were to show 01:42PM that the application efficiencies between samples were identical?
A No, I don't.
Q Okay. Would you agree with me that DNA derived from different materials will replicate with 01:43PM different efficiencies?
A DNA, so derived from different materials -- can you give me an example?
Q For instance, DNA from a water sample as opposed to DNA from a soil sample, is it possible 01:43PM that they would replicate with different efficiencies or would they all reflect the same efficiency?
A It is possible that you would have different efficiency. 01:43PM

Q Okay, and back to the question I started. Do you know whether any controls were put in place to measure any differential in replication efficiency?

A To the best of my knowledge we didn't have any controls, like that one. We did have inhibition controls, so we always ran a spike to make sure there was no inhibition in the sample. 01:43PM

Q Okay. Was -- you used the term thermocycler?

A Uh-huh.

Q Okay. Is that the same or different from a spectrophotometer? 01:44PM

A That's different.

Q That's different, okay. Was a spectrophotometer used?

A The spectrophotometer -- 01:44PM

Q Thank you.

A -- is used to quantify the starting amount of DNA, and so that's shown in that DNA column in the spreadsheet.

Q Okay. That's the column labeled just DNA? 01:44PM

A Yes, nanograms per liter.

Q A question on that quickly. On the first page between two-thirds of the way down there's a negative number. I think you testified about what that means before.

A Yes.
Q Negative 1.5, why is that a negative number?
A It means that it's the amount DNA that was -- in that sample was below the detection of the spectrophotometer. 01:44PM
Q Is that -- should that be treated at same as a zero or is it substantively different?
A Technically in the spreadsheet that should read less than and then it should be the detection limit for the spectrophotometer. That's technically how it should be in there. 01:45PM
Q Okay. Do you know what the detection limit was on North Wind's equipment?
A For this -- no, I don't know what the detection limit is for that spectrophotometer. Usually it's around a nanogram per liter or less actually, tenths of nanograms per liter. 01:45PM
Q Do you know whether it was calibrated to an NIST standard?
A No, I don't know that. 01:45PM
Q Okay. What was the percent CV, coefficient of variation?
A For the DNA quantification?
Q For the spectrophotometer.
A I don't know, but the spec is only being used 01:45PM

to establish the amount of template DNA.
Q Did you at any point ask North Wind for this information?
A No.
Q Let's look at Figure 5 and Figure 6 to your report, and these are maps of the watershed showing location is of qPCR testing. Are you there? 01:45PM
A Yep.
Q Okay. Look at Figure 6 for me. Why -- and these reflect -- according to your title, these are soil sample locations? 01:46PM
A Correct.
Q Why are the soil sample locations relatively clustered?
A I believe that that was due to the places where CDM was able to collect soil samples, but I don't know further than that. 01:46PM
Q Okay. On neither map -- neither Figure 5 nor Figure 6 includes the results when they came back as below the detection limit? 01:47PM
A Correct.
Q Why did you elect not to include those?
A It would have made the map very, very, very hard to read.
Q So aesthetics? 01:47PM

A Yes. I mean it's easier just to show the ones -- we already know how many were below the detection limits, so it's easier just to show the ones that were more clear as you said.

Q Okay. On Figure 5, why did you not elect to do additional water samples lower down in terms of altitude in the watershed? 01:47PM

A The sampling was focused around the poultry houses, and that was -- the sampling plan, again, was to show the transport of the or the gradient of the pollution from the edge of the field or from the field to the edge of the field and then out into the waters, and so a lot of the sampling was focused up in the area where there was more poultry houses. 01:47PM

Q Why were no tests run between January and April? 01:48PM

MR. PAGE: Object to the form.

Q Let's go back to the packet of North Wind results that I gave you earlier. I'm not sure which exhibit it is. I think it's this one here. That's Exhibit 11. Flip through that packet to the date on Set 3 right there. 01:48PM

A This is Set 4.

Q I'm sorry. That's Set 4.

A 1-21-8. 01:49PM

Q Okay, and then Set 4 is in April; is that right?
A Correct.
Q Okay. Why did you -- why were no tests done between January and April? 01:49PM
MR. PAGE: Object to the form.
A I can only speculate, but that was about the time when we were getting ready for the preliminary injunction, so I would think that they had finished up one set of samples and were waiting for guidance 01:49PM on the next set to go forward.
Q Was it the State's intention originally to test all 550 samples that were sent to North Wind?
A No. As I remember those conversations, the intention was to over collect samples and then based 01:49PM on the distribution that we obtained throughout the watershed, that we might then test some subset of those. That's my recollection.
Q I apologize for this not being stapled. Exhibit No. 16 is an E-mail from Jennifer Weidhaas 01:50PM to Kate Field at Oregon State, and you are copied on it, and in the second sentence -- I'm sorry, the third sentence she writes, we are in the final stages of optimizing the protocol before we test out the 500 or so samples we have achieved. Why would 01:50PM

Jennifer Weidhaas think --

MR. PAGE: Object to the form.

Q -- that the intention was to test all 500 samples?

MR. PAGE: Object to the form. 01:50PM

A I don't think she thought that. I think she was just saying they had 500 or so samples.

Q Okay. Let's go back to the PCR process. If I understand correctly from your testimony at the hearing -- well, I'm going to ask about the gene 01:51PM copy detection limit for the process, and if I understand your testimony from the hearing, you said it was 2,000 gene copies to quantify; does that sound right?

A 2,000 gene copies per liter. 01:51PM

Q Okay. That's important. Per liter to quantify, and then it was 6 gene copies per gram in solid matter to identify presence-absence. Is that correct?

A No. 6 microliters or, sorry, 6 copies per 01:51PM microliter in a PCR assay.

Q Okay. Then you testified that it was 50 or so for water; is that correct?

A In one assay. So there's a big difference -- I've got to kind of explain this. 01:51PM

Q Please do.
A You have a little test tube and you are saying, okay, I can detect 6 copies in this little test tube, that's one assay, but that's not really so relevant to an environmental sample. So you go out and get an environment sample and you say, okay, in this big sample that I have to concentrate down onto a filter and then extract from the filter, how many copies do I need to go from -- to detect from this big volume, so 2,000 copies per liter concentration-wise is the same as two copies per microliter, but it's simply that you are concentrating it down. That's the difference between saying you can detect a very small number in the test tube versus in this big volume, it's going to take a much larger number because now you are in a liter of water and you've diluted the sample. 01:52PM 01:52PM 01:52PM
Q Why is -- is the difference there a function of the process by which the sample is reduced to a testable form? 01:52PM
A That's a part of it, yes, because of the fact that you are concentrating large volume to small volume. Then you are alluding it -- you're getting it off of that filter and then you are extracting the DNA. So each of those processes has some 01:53PM

inefficiency associated with it.
Q Okay.
A Really for an environmental sample being able to concentrate or to detect 2,000 copies per liter is good. 01:53PM
Q Your testimony, as I understand it, is that the PCR sequence, the actual DNA, correlates with indicator bacteria?
A In the litter.
Q In the litter. In the litter, and it 01:53PM correlates with more strongly with Enterococci than E. coli; is that correct?
A Correct.
Q I want to walk you through the process of developing the correlation just to make sure I 01:53PM understand it. So you calculated the correlation between gene copies of the PCR sequence and number of Enterococci?
A Can you repeat that to make sure?
Q Sure. It's the same question I just asked 01:54PM you, which is you developed a correlation between the PCR sequence and the Enterococci?
A In poultry litter samples, contaminated poultry litter samples.
Q Right. How many samples did you use to base 01:54PM

your correlation on?
A All 10 of the litter samples that we had at the time I did the correlations.
Q Okay, and do you recall the R squared value?
A It's in my report. 01:54PM
Q Okay.
A It would be .74.
Q Did you calculate a P value?
A Yeah. .0013.
Q Okay, and what was the nature of the 01:55PM relationship?
A Positive linear.
Q Okay, and now the same questions for E. coli. How many samples did you use?
A The same, the 10 samples. 01:55PM
Q Okay, and what was the R squared value?
A Let me look in my report.
Q Sure.
A It was about .35, but I want to make sure that I'm accurate. For E. coli, R squared equals .395 01:55PM and P equals 0.052.
Q Thank you, and what was the relationship there?
A That was also positive.
Q Did you calculate a correlation between the 01:55PM

PCR sequence and indicator bacteria in field soil where litter was land applied?

A No, I did not do that.

Q Okay. Did you calculate the correlation in edge of field samples? 01:56PM

A Between edge of field samples and what?

Q I'm sorry. Between -- in edge of field samples did you calculate a correlation between the PCR sequence and indicator bacteria?

A No, I did not. 01:56PM

Q Okay. Did you do it in surface water?

A No, I did not.

Q Okay. Did you do it in groundwater?

A No, I did not.

Q Did you do it for springs? 01:56PM

A Nope.

Q For wells?

A No.

Q Okay. Go back, if you would, to the few pages I gave you from your journal article you submitted. 01:56PM I forget what exhibit number it was. It was pretty early on.

MS. SOUTHERLAND: Exhibit 2.

Q Exhibit 2.

A All right. 01:57PM

Q If you go to the very last page, can you tell me what this Page 29 -- can you tell me what this chart represents?
A This is the correlation between the biomarker and indicator organisms in water samples. These are the water samples that were done for the -- that were analyzed for the paper. So we have log E. coli or Enterococcus on the Y axis and log biomarker on the X axis. 01:57PM
Q Now, you say these are the samples that were done for the paper. Are these samples from the IRW? 01:58PM
A These are samples from the IRW.
Q Are these samples that were tested as part of the State's case?
A Yes, they are. 01:58PM
Q Are these samples included in your data report?
A Yes, they are.
Q Let me take you through the same questions I asked you before. For the correlation between Enterococci and the PCR sequence, what was the R squared? 01:58PM
A 0.89.
Q And what was the P value, if you calculated one? 01:58PM

A I don't have a P value. There's no P value on this graph. I would have to go back through the paper and look at the P value.

Q Okay, and do you recall the nature of the relationship? 01:58PM

A Positive linear.

Q And for E. coli --

A It is.

Q -- R squared?

A R squared is 0.85. 01:58PM

Q And do you recall the P value?

A I don't recall the P value.

Q Was the relationship linear and positive again or positive linear?

A Positive and linear. 01:59PM

Q Okay. In order for the PCR sequence to be an indicator for indicator bacteria derived from poultry, should the correlation between the PCR sequence and the indicator bacteria be consistent throughout the various stages of the pathway that 01:59PM you were looking at?

A Well, the PCR biomarker is an indicator of poultry fecal contamination. It's not an indicator of indicators. We don't need an indicator of indicators. It's an indicator of poultry fecal 01:59PM

contamination.
Q Okay, but in order for it to be an indicator of poultry fecal contamination, is it necessary that the PCR sequence share the same fate and transport as pathogens from poultry litter? 02:00PM
A Can you say that again? I just got to get the first part.
Q Sure. In order for it to be an indicator -- you've just said it is an --
A Indicator of poultry fecal contamination. 02:00PM
Q Right, and that fecal contamination you are talking about here is bacteria; correct?
A Correct.
Q Okay. So in order for the presence of the indicator -- 02:00PM
A I'm sorry. Let me go back there because we're not only concerned about bacterial fecal contamination from poultry, we're also concerned about nutrient contamination. So we can add nutrients and metals to that list. 02:00PM
Q We'll talk about -- let's table the nutrients and the metals for just a second and let's talk about bacteria. In order for it to indicate the presence of bacteria derived from poultry, is it necessary that the PCR -- that the Brevibacterium 02:00PM

that you identified share the fate and transport characteristics of other bacteria from poultry litter?

A It would have to have certain fate and transport characteristics in common. 02:01PM

Q Okay. If we compare the correlations that we discussed here, so the correlation, let's say, taking Enterococcus, for instance, the relationship between Enterococcus and the sequence in litter as .75 and the relationship between Enterococcus and 02:01PM the biomarker -- the sequence in water is .89, which is different; correct?

A It's different, but it's certainly within the bounds of what you would expect from regular sampling error. 02:01PM

Q Okay. How big a difference can you have within the bounds of regular sampling error?

A In environmental microbiology we're very happy to get correlations of .3 as long as they're statistically significant, even .2 sometimes. So 02:01PM there's a really wide range of what you can get from correlations and still be biologically meaningful.

Q Okay. So does it surprise you at all then that the correlation that you got between E. coli and the PCR sequence in litter was .39 you told me 02:02PM

and in water it's .85?
A No, that doesn't surprise me.
Q It doesn't surprise you that they're much more correlated in water than they are in litter?
A No. I mean both of those correlations are done on a relatively small sample size, and the other issue with the dataset is that the data for both Enterococcus and E. coli are truncated, which means they kind of are cut off at the high end, so that's going to make the correlation not as complete, not as accurate as it could be. 02:02PM
Q I'm sorry. Tell me what you mean by that, that it's cut off at the high end.
A So sometimes with the indicator bacteria samples, the lab would dilute the sample out to the point where they could detect 12,000 or they could quantify 12,000 but no higher simply because they didn't dilute the sample out enough to be able to detect a higher number, and so that gives you what is called a truncated dataset, where you have it cut off at the high end because you simply couldn't measure the samples any higher than 12,000. So it's really not surprising that the correlations will vary but, you know, really to see -- in environmental samples to see correlations like that 02:03PM

at all is very encouraging and would not be likely at all to be the result of a chance event.

Q Okay. You mentioned statistical significance. What is the relevance of statistical significance to relying on the correlation here? 02:03PM

A So when you look at a correlation, you take several parameters into account, but the first one that you would look at is the P value and that would be the statistical significance of the result and if P is less than 0.05, then by most general 02:04PM statistical cut-offs, then that's a statistically significant correlation. It means that if you repeated that experiment 100 times, 95 percent of the time you would still get some sort of a correlation between the variables. That's what that 02:04PM 0.05 means.

Then you have the R squared. The R squared value actually tells you to what extent the variables co-vary. So if R squared is close to 1, then they co-vary tightly. If R squared is lower, 02:04PM then there's more variability in their relationship to each other.

Q Okay. Taking the litter samples, is it your testimony that based on the 10 samples here and the correlation that you developed, that if you took any 02:05PM

10 samples from anywhere in the watershed, you would expect to find these same relationships?

A I would expect to find similar relationships, not necessarily the same R squared, but I would expect to find a relationship between indicator bacteria concentrations and the biomarker. 02:05PM

Q Okay. Did you perform any calculations as to how many litter samples you should take to accurately characterize the watershed?

A No. 02:05PM

Q In the water samples -- background question. Poultry is not the only source of indicator bacteria in surface water in the IRW; correct?

A Poultry is a dominant source of indicator bacteria in the watershed. 02:05PM

Q I knew you believed that, but there are other sources of indicator bacteria?

A There can be.

Q There can be?

A Yes. 02:05PM

Q Okay. Are there?

A Okay.

Q Do you think it's possible that poultry is the only source of indicator bacteria in the IRW?

A Again, poultry are a dominant source but it is 02:06PM

possible that there are other sources.
Q Well, if they're a dominant source, then there must be other sources. Can we agree there are other sources?
A I can agree that there are other sources, yes. 02:06PM
Q Thank you. What -- when you did the correlation here for your paper between PCR sequence and indicator bacteria in the water, did you perform any -- did you do anything to control for ultimate sources of the indicator bacteria? 02:06PM
A We measured the poultry litter biomarker, but we did not have specific microbial source tracking tests for any other species.
Q Okay, and so the Enterococcus and the E. coli that are included in this calculation, the 02:06PM correlation in the water, those include all indicator bacteria or all E. coli and all Enterococcus regardless of source?
A That would include all E. coli and all Enterococci that were culturable. 02:07PM
Q Okay. Did you find the PCR sequence in all of your edge of field samples?
A No. I don't think --
Q You can probably look on Exhibit 12 and it will tell you. 02:07PM

A Thank you. I know it was quantifiable in 16 of them, but -- so there are several here, one, two, three, four in which it is below detection limit.

Q Okay. What does -- this is a terminology question. What does EOF SPREAD mean as distinct from the samples at the top which are just EOF; do you know the sample naming? 02:08PM

A You know, I was actually always confused about that. I had to go and ask CDM every time I was looking at the samples, so I don't know. 02:08PM

Q Okay. So you don't remember the answer. Would it surprise you to not find -- to not find the PCR sequence in edge of field samples?

A In some cases I know that the litter spreading had occurred some weeks or months prior to the sampling. So with that knowledge, I'm not surprised that we don't find it sometimes. 02:08PM

Q If you look at the numbers of gene copies identified at the beginning of the first page of Exhibit 12 here in the edge of field samples, they range from to the 4th up to the 7th; do you see that? 02:09PM

A I see that.

Q Does that spread surprise you at all?

A No, again, because depending on the amount of 02:09PM

litter that was spread and the amount of -- or the time since spreading, the amount of rainfall that occurred, all of those things could influence the numbers a lot.

Q Okay. We talked about the difference between the correlation in the litter and the correlation in the water and how for both Enterococci and E. coli the correlation is actually better than it is in the litter. 02:09PM

A It's closer to one. 02:09PM

Q Right. It's stronger; is that a fair characterization?

A You could -- the sample size is smaller with the water samples, so you have to take that with a grain of salt. 02:10PM

Q Okay. Given that grain of salt, what could happen between litter and water to make the correlation stronger?

A With that many samples, it could be just stochastic chance variability. Recall there's four water samples there and there's ten litter samples, so that could certainly just be varying out of -- the variability could be just sampling error. 02:10PM

Q Okay. Did you calculate any correlation between the PCR sequence and any nutrient? 02:10PM

A You asked me if I had correlations between nutrients and PCR?
Q And the PCR sequence?
A I didn't did any such calculations.
Q Okay. Can you calculate such a correlation between the PCR sequence and any other component of Dr. Olsen's PCA? 02:11PM
A I did not.
Q Okay. In forming your conclusions in this case, did you rely at all on Dr. Engel's work? 02:11PM
A I relied on his modeling work to the extent that I utilized the numbers for the amounts of fecal material contributed by the poultry litter.
Q How about Dr. Wells' modeling work?
A Dr. Wells'? Not to my knowledge. 02:11PM

MR. TODD: I'm done.

MR. GRAVES: I have no questions.

MS. LONGWELL: I may have a few.

VIDEOGRAPHER: We're now off the Record. The time is 2:12 p.m. 02:12PM

(Following a short recess at 2:12 p.m., proceedings continued on the Record at 2:23 p.m.)

VIDEOGRAPHER: We are back on the Record. The time is 2:23 p.m.

DIRECT EXAMINATION

BY MS. LONGWELL:

Q Dr. Harwood, my name is Nicole Longwell and I'm counsel for Peterson Farms, and I've got some questions for you, and they're going to be a bit like shooting a shotgun and it's going to be all over the place, and I apologize for that, but because I'm following up, that's the nature of the beast. 02:23PM

Let me start with first asking you some questions about your review of material provided to you by North Wind. Can you describe the process you went through when you received like a -- let me be specific -- like a QA/QC review of yours that you had when you received their sampling results? 02:24PM

A So when I would receive their sampling results, I would first, of course, read over and make sure that we were -- that I knew what the samples entailed, and then I would look through and see if there were any anomalies like, for example, not applicable where it shouldn't be or a no where there should have been a yes, and then basically just go through the results and take a look at them. Since I had already reviewed their SOPs, then I'm comfortable with their operating procedures throughout the project. 02:24PM 02:25PM

Q Did you -- in your review, did you review whether or not the units measured matched the media that was identified on the sampling results?
A I would generally do that, but I have to admit that sometimes in being, what, in a hurry, as people usually are or sometimes are, then I would just scan down the list of the figures and not say, okay, is this exactly the correct unit. 02:25PM
Q I'm sorry. Go ahead.
A I was going to say that I know at least on one of the reports we had to revise some units, and that was something that, you know, that I caught later on. 02:25PM
Q Okay, and when you say revised, did you send it back to North Wind or did you revise in your office some of the data that North Wind sent? 02:25PM
A No, no, I never revise anything in my office. Anything that was revised was done -- we would talk about it and then the revision would be made and it would be sent out to everybody. 02:26PM
Q So they would resend -- if you found something wrong where they put a non-applicable when there should have been something there, you would contact North Wind and have them reissue the result?
A Correct. 02:26PM

Q So if you identified a change, was there any time when you didn't ask North Wind to change the sample result but just merely changed it within your report?

A I would -- I never changed anything in my report. Everything was always changed at the level of North Wind and then distributed to the whole team. 02:26PM

Q Okay. When you received the results from North Wind, did you receive sort of an entire package with each of the sample results, which included like their testing, you know, their blank testing and QA/QC that they did? 02:26PM

A No, I didn't receive individual QA/QC results. So generally the transmission would be electronic, and I would get a list of the samples that had been processed and the results and, again, having already reviewed the QA/QC and knowing how attentive they are to details, then that was sufficient. 02:27PM

Q So you relied upon the SOPs that they put in place? 02:27PM

A Correct.

Q And that they had -- and assumed that they had instituted those with processing every sample?

A Correct. 02:27PM

Q Let me have you look at Exhibit 1, which is your report, Page 27, which is Table 5.
A All right.
Q The table is identified as qPCR results for litter, soil and water samples with quantifiable concentrations on the poultry litter biomarker. Does this table include the samples where you did not detect the biomarker? 02:28PM
A This sample does not include or this table does not include samples where the biomarker was not detected. In fact, it only includes samples where the concentration was high enough to be quantifiable. 02:28PM
Q Did you ever prepare a Table 5 for your report that included samples that where the biomarker was non-detectable? 02:28PM
A Not for this report.
Q Have you prepared it for another report?
A Wow. I'd have to look back at that old report, but I don't recall preparing one like that. 02:28PM
Q So are you assuming -- I may not assume. Did you prepare it in preparation of a draft report?
A I had -- I certainly had spreadsheets that have all the results in it. In fact, one of them was shown here today. 02:29PM

Q But did you ever prepare a Table 5 that included --

MR. PAGE: I'll object to the form. Table 5 says samples quantifiable concentrations. It would be kind of foolish to put on a quantifiable concentration table results that are not quantifiable and not even present. 02:29PM

MS. LONGWELL: I understand your objection, but I would still like the witness to answer the question. 02:29PM

Q Have you ever prepared a Table 5 for a draft report or previously that included samples that were -- had a non-detect for the biomarker?

MR. PAGE: Object to the form.

A I can't specifically remember, but I don't recall doing that. 02:29PM

Q Have you undertaken any efforts to determine what effect the chemicals and properties of the soils and water in the Illinois River watershed may have on this poultry litter biomarker? 02:30PM

A The tests that we have done on the soils and water would be detection and a quantification of the biomarker. Does that answer your question?

Q No. Actually the question was, have you conducted any tests to determine -- let's start 02:30PM

back. Have you determined what effects the chemicals and properties of the soils in the Illinois River watershed may have on the poultry litter biomarker?

A No. 02:30PM

Q Have you studied or done any testing as to whether the chemicals or properties in the water within the Illinois River watershed would affect -- those chemicals and properties may have on the poultry litter biomarker? 02:30PM

MR. PAGE: Object to the form.

A Again, we've simply sampled the waters and determined the concentration, but there's been no attempt to correlate the chemistry with the concentration of the biomarker or how that might affect it. 02:31PM

Q Have you conducted any tests to see what effect pH within the soils may have on the poultry litter biomarker?

A We've not conducted any systematic tests to determine the relationship between pH and the biomarker, no. 02:31PM

Q Have you done any testing with regards to the effect of pH in water on the poultry litter biomarker? 02:31PM

A No, we have not.
Q Do you know what the range of pH is in the soils within the IRW or the Illinois River watershed?
A Not off the top of my head, no. 02:31PM
Q Have you done any research into what the pH levels in the soils within the Illinois River watershed is?
A No.
Q Have you conducted any tests or are you aware 02:31PM of what the pH level in the waters within the Illinois River watershed are?
A I've looked at the data that has been collected on the water pH and don't recall seeing any strange ranges far from 7, but specifically, no, 02:32PM I haven't systematically studied that.
Q So can you identify what the range of pH is in the waters in the Illinois River watershed?
A No, I can't.
Q Have you tested to see if there any other 02:32PM chemical compounds within the Illinois River watershed that may destroy or alter the poultry litter biomarker?
A No, I have not.
Q Have you conducted any tests or studied how 02:32PM

the poultry litter biomarker moves within the underground water formation in the Illinois River watershed?

A So the only testing that we've done is the sampling of the biomarker in some of these subsurface compartments but -- so just simply the testing in the subsurface waters. 02:33PM

Q Have you done any testing to determine whether or not the chemistry in the rocks within these underground water formations has any effect or alters the poultry litter biomarker in any way? 02:33PM

A No.

Q Let me have you look at Exhibit 12. This is my understanding, but the sample prefix LAL means land application, that the land application sites where the soil was tested; is that your understanding of what those samples are? 02:33PM

A That's generally correct. There are some -- the LAL samples are -- most of them are soil but not all of them. 02:34PM

Q Okay. In fact, outside of them, the matrix is identified on Exhibit 12, too; is that correct?

A That's correct.

Q Looking solely at the soil samples, can you identify any of the soil samples on Exhibit 12 as 02:34PM

being soil samples from a Peterson contract grower's farm?

A No, I can't.

Q Can you identify the soil samples listed on Exhibit 12 as being from any contract grower for any of the defendants in this case? 02:34PM

A I don't have any knowledge of which samples correspond to which of the growers.

Q What about with regards to the litter; can you identify which of the samples go with which specific defendant contract grower? 02:34PM

A Not off the top of my head, although that data is available, but I can't do it right here.

Q With regard to the edge of field samples, the EOF and the EOF SPREAD samples, could you identify which properties and which property owners those samples were taken adjacent to? 02:35PM

A Do you mean could I do that right now?

Q Well, do you know?

A No. Do I -- 02:35PM

Q Do you have that information within your files?

A I believe I have it in my files, but I know I could obtain it from CDM if I needed to get it.

Q With regard to your findings of the biomarker 02:35PM

in the water samples within the Illinois River watershed, can you specifically trace back that biomarker to any particular defendants' contract growers farm?

A Could you repeat that? 02:35PM

Q Sure. Actually I may just have the court reporter repeat it.

(Whereupon, the court reporter read back the previous question.)

A I think that would be possible to do within 02:36PM the soil samples and edge of field samples, but once it had got farther away, then it's going to be potentially generalized contributions from a lot of different places. So then once it's out in the surface water or the groundwater, I don't see how it 02:36PM could be traced back to a specific grower, considering that there's a lot of different ones in the watershed.

Q So the answer is, no, you could not do that?

A Except I think, again, in the soil sample or 02:36PM an edge of field sample if it was associated with a grower.

Q But the question was with regards to water. So with regards to the water samples, you cannot specifically identify which -- the poultry litter 02:37PM

biomarker, which defendants' contract farmer it came from?

A I understand your question, and the edge of field samples are technically water samples. So except for the edge of field samples, then the answer would be no. 02:37PM

MS. LONGWELL: I don't have any further questions. Do you?

MR. GRAVES: No.

MS. LONGWELL: Thank you, Dr. Harwood. 02:37PM

MR. PAGE: We have no cross examination.

VIDEOGRAPHER: This concludes the deposition of Valerie Harwood.

MR. BULLOCK: She'll read and sign.

VIDEOGRAPHER: We're now off the Record, the time is 2:37 p.m. 02:37PM

(Whereupon, the deposition was concluded at 2:37 p.m.)

SIGNATURE PAGE

I, Valerie Harwood, PhD, do hereby certify that the foregoing deposition was presented to me by Lisa A. Steinmeyer as a true and correct transcript of the proceedings in the above styled and numbered cause, and I now sign the same as true and correct.

WITNESS my hand this _____ day of _____, 2008.

_____

VALERIE HARWOOD, PhD

SUBSCRIBED AND SWORN TO before me this _____ day of _____, 2008.

_____

Notary Public

My Commission Expires:

_____

02:37PM

C E R T I F I C A T E

STATE OF OKLAHOMA)
) ss.
COUNTY OF TULSA)

I, Lisa A. Steinmeyer, Certified Shorthand Reporter within and for Tulsa County, State of Oklahoma, do hereby certify that the above named witness was by me first duly sworn to testify the truth, the whole truth and nothing but the truth in the case aforesaid, and that I reported in stenograph her deposition; that my stenograph notes were thereafter transcribed and reduced to typewritten form under my supervision, as the same appears herein.

I further certify that the foregoing 171 pages contain a full, true and correct transcript of the deposition taken at such time and place.

I further certify that I am not attorney for or relative to either of said parties, or otherwise interested in the event of said action.

WITNESS MY HAND AND SEAL this 25th day of July, 2008.

_____

LISA A. STEINMEYER, CRR
CSR No. 386

CORRECTIONS TO THE DEPOSITION OF

VALERIE HARWOOD, PhD

PAGE AND LINE NUMBER	CORRECTION